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(54) Nucleic acid encoding a human protein phosphatase

(57) The present invention relates to nucleic acids encoding a novel human protein phosphatase of the family of protein serine/threonine phosphatases. In particular, it relates to novel DNA sequences encoding serine/threonine protein phosphatase, to expression plasmids containing said nucleic acids, to host organisms transformed by said expression plasmids, to the production of said protein by culturing said transformant, to antibodies specifically binding to said phosphatase and to agonists and/or antagonists for said protein, and to antisense MP-19 nucleic acid. Furthermore, the invention relates to serine or threonine residues and epitopes comprising said residues dephosphorylated by said protein and pharmaceutical compositions comprising said protein or agonists or antagonists thereof for the treatment of diseases influenced by changes in phosphorylation which controls e.g. cell proliferation and/or differentiation, to diagnostic kits and to *in vitro* diagnostic methods for the detection of phosphorylation dependent diseases such as e.g. cancer.

Fig. 1

511-512	513-514	515-516	517-518	519-520	521-522	523-524	525-526	527-528	529-530	531-532	533-534	535-536	537-538	539-540	541-542	543-544	545-546	547-548	549-550	551-552	553-554	555-556	557-558	559-560	561-562	563-564	565-566	567-568	569-570	571-572	573-574	575-576	577-578	579-580	581-582	583-584	585-586	587-588	589-590	591-592	593-594	595-596	597-598	599-600	601-602	603-604	605-606	607-608	609-610	611-612	613-614	615-616	617-618	619-620	621-622	623-624	625-626	627-628	629-630	631-632	633-634	635-636	637-638	639-640	641-642	643-644	645-646	647-648	649-650	651-652	653-654	655-656	657-658	659-660	661-662	663-664	665-666	667-668	669-670	671-672	673-674	675-676	677-678	679-680	681-682	683-684	685-686	687-688	689-690	691-692	693-694	695-696	697-698	699-700	701-702	703-704	705-706	707-708	709-710	711-712	713-714	715-716	717-718	719-720	721-722	723-724	725-726	727-728	729-730	731-732	733-734	735-736	737-738	739-740	741-742	743-744	745-746	747-748	749-750	751-752	753-754	755-756	757-758	759-760	761-762	763-764	765-766	767-768	769-770	771-772	773-774	775-776	777-778	779-780	781-782	783-784	785-786	787-788	789-790	791-792	793-794	795-796	797-798	799-800	801-802	803-804	805-806	807-808	809-810	811-812	813-814	815-816	817-818	819-820	821-822	823-824	825-826	827-828	829-830	831-832	833-834	835-836	837-838	839-840	841-842	843-844	845-846	847-848	849-850	851-852	853-854	855-856	857-858	859-860	861-862	863-864	865-866	867-868	869-870	871-872	873-874	875-876	877-878	879-880	881-882	883-884	885-886	887-888	889-890	891-892	893-894	895-896	897-898	899-900	901-902	903-904	905-906	907-908	909-910	911-912	913-914	915-916	917-918	919-920	921-922	923-924	925-926	927-928	929-930	931-932	933-934	935-936	937-938	939-940	941-942	943-944	945-946	947-948	949-950	951-952	953-954	955-956	957-958	959-960	961-962	963-964	965-966	967-968	969-970	971-972	973-974	975-976	977-978	979-980	981-982	983-984	985-986	987-988	989-990	991-992	993-994	995-996	997-998	999-1000	1001-1002	1003-1004	1005-1006	1007-1008	1009-1010	1011-1012	1013-1014	1015-1016	1017-1018	1019-1020	1021-1022	1023-1024	1025-1026	1027-1028	1029-1030	1031-1032	1033-1034	1035-1036	1037-1038	1039-1040	1041-1042	1043-1044	1045-1046	1047-1048	1049-1050	1051-1052	1053-1054	1055-1056	1057-1058	1059-1060	1061-1062	1063-1064	1065-1066	1067-1068	1069-1070	1071-1072	1073-1074	1075-1076	1077-1078	1079-1080	1081-1082	1083-1084	1085-1086	1087-1088	1089-1090	1091-1092	1093-1094	1095-1096	1097-1098	1099-1100	1101-1102	1103-1104	1105-1106	1107-1108	1109-1110	1111-1112	1113-1114	1115-1116	1117-1118	1119-1120	1121-1122	1123-1124	1125-1126	1127-1128	1129-1130	1131-1132	1133-1134	1135-1136	1137-1138	1139-1140	1141-1142	1143-1144	1145-1146	1147-1148	1149-1150	1151-1152	1153-1154	1155-1156	1157-1158	1159-1160	1161-1162	1163-1164	1165-1166	1167-1168	1169-1170	1171-1172	1173-1174	1175-1176	1177-1178	1179-1180	1181-1182	1183-1184	1185-1186	1187-1188	1189-1190	1191-1192	1193-1194	1195-1196	1197-1198	1199-1200	1201-1202	1203-1204	1205-1206	1207-1208	1209-1210	1211-1212	1213-1214	1215-1216	1217-1218	1219-1220	1221-1222	1223-1224	1225-1226	1227-1228	1229-1230	1231-1232	1233-1234	1235-1236	1237-1238	1239-1240	1241-1242	1243-1244	1245-1246	1247-1248	1249-1250	1251-1252	1253-1254	1255-1256	1257-1258	1259-1260	1261-1262	1263-1264	1265-1266	1267-1268	1269-1270	1271-1272	1273-1274	1275-1276	1277-1278	1279-1280	1281-1282	1283-1284	1285-1286	1287-1288	1289-1290	1291-1292	1293-1294	1295-1296	1297-1298	1299-1300	1301-1302	1303-1304	1305-1306	1307-1308	1309-1310	1311-1312	1313-1314	1315-1316	1317-1318	1319-1320	1321-1322	1323-1324	1325-1326	1327-1328	1329-1330	1331-1332	1333-1334	1335-1336	1337-1338	1339-1340	1341-1342	1343-1344	1345-1346	1347-1348	1349-1350	1351-1352	1353-1354	1355-1356	1357-1358	1359-1360	1361-1362	1363-1364	1365-1366	1367-1368	1369-1370	1371-1372	1373-1374	1375-1376	1377-1378	1379-1380	1381-1382	1383-1384	1385-1386	1387-1388	1389-1390	1391-1392	1393-1394	1395-1396	1397-1398	1399-1400	1401-1402	1403-1404	1405-1406	1407-1408	1409-1410	1411-1412	1413-1414	1415-1416	1417-1418	1419-1420	1421-1422	1423-1424	1425-1426	1427-1428	1429-1430	1431-1432	1433-1434	1435-1436	1437-1438	1439-1440	1441-1442	1443-1444	1445-1446	1447-1448	1449-1450	1451-1452	1453-1454	1455-1456	1457-1458	1459-1460	1461-1462	1463-1464	1465-1466	1467-1468	1469-1470	1471-1472	1473-1474	1475-1476	1477-1478	1479-1480	1481-1482	1483-1484	1485-1486	1487-1488	1489-1490	1491-1492	1493-1494	1495-1496	1497-1498	1499-1500	1501-1502	1503-1504	1505-1506	1507-1508	1509-1510	1511-1512	1513-1514	1515-1516	1517-1518	1519-1520	1521-1522	1523-1524	1525-1526	1527-1528	1529-1530	1531-1532	1533-1534	1535-1536	1537-1538	1539-1540	1541-1542	1543-1544	1545-1546	1547-1548	1549-1550	1551-1552	1553-1554	1555-1556	1557-1558	1559-1560	1561-1562	1563-1564	1565-1566	1567-1568	1569-1570	1571-1572	1573-1574	1575-1576	1577-1578	1579-1580	1581-1582	1583-1584	1585-1586	1587-1588	1589-1590	1591-1592	1593-1594	1595-1596	1597-1598	1599-1600	1601-1602	1603-1604	1605-1606	1607-1608	1609-1610	1611-1612	1613-1614	1615-1616	1617-1618	1619-1620	1621-1622	1623-1624	1625-1626	1627-1628	1629-1630	1631-1632	1633-1634	1635-1636	1637-1638	1639-1640	1641-1642	1643-1644	1645-1646	1647-1648	1649-1650	1651-1652	1653-1654	1655-1656	1657-1658	1659-1660	1661-1662	1663-1664	1665-1666	1667-1668	1669-1670	1671-1672	1673-1674	1675-1676	1677-1678	1679-1680	1681-1682	1683-1684	1685-1686	1687-1688	1689-1690	1691-1692	1693-1694	1695-1696	1697-1698	1699-1700	1701-1702	1703-1704	1705-1706	1707-1708	1709-1710	1711-1712	1713-1714	1715-1716	1717-1718	1719-1720	1721-1722	1723-1724	1725-1726	1727-1728	1729-1730	1731-1732	1733-1734	1735-1736	1737-1738	1739-1740	1741-1742	1743-1744	1745-1746	1747-1748	1749-1750	1751-1752	1753-1754	1755-1756	1757-1758	1759-1760	1761-1762	1763-1764	1765-1766	1767-1768	1769-1770	1771-1772	1773-1774	1775-1776	1777-1778	1779-1780	1781-1782	1783-1784	1785-1786	1787-1788	1789-1790	1791-1792	1793-1794	1795-1796	1797-1798	1799-1800	1801-1802	1803-1804	1805-1806	1807-1808	1809-1810	1811-1812	1813-1814	1815-1816	1817-1818	1819-1820	1821-1822	1823-1824	1825-1826	1827-1828	1829-1830	1831-1832	1833-1834	1835-1836	1837-1838	1839-1840	1841-1842	1843-1844	1845-1846	1847-1848	1849-1850	1851-1852	1853-1854	1855-1856	1857-1858	1859-1860	1861-1862	1863-1864	1865-1866	1867-1868	1869-1870	1871-1872	1873-1874	1875-1876	1877-1878	1879-1880	1881-1882	1883-1884	1885-1886	1887-1888	1889-1890	1891-1892	1893-1894	1895-1896	1897-1898	1899-1900	1901-1902	1903-1904	1905-1906	1907-1908	1909-1910	1911-1912	1913-1914	1915-1916	1917-1918	1919-1920	1921-1922	1923-1924	1925-1926	1927-1928	1929-1930	1931-1932	1933-1934	1935-1936	1937-1938	1939-1940	1941-1942	1943-1944	1945-1946	1947-1948	1949-1950	1951-1952	1953-1954	1955-1956	1957-1958	1959-1960	1961-1962	1963-1964	1965-1966	1967-1968	1969-1970	1971-1972	1973-1974	1975-1976	1977-1978	1979-1980	1981-1982	1983-1984	1985-1986	1987-1988	1989-1990	1991-1992	1993-1994	1995-1996	1997-1998	1999-2000	2001-2002	2003-2004	2005-2006	2007-2008	2009-2010	2011-2012	2013-2014	2015-2016	2017-2018	2019-2020	2021-2022	2023-2024	2025-2026	2027-2028	2029-2030	2031-2032	2033-2034	2035-2036	2037-2038	2039-2040	2041-2042	2043-2044	2045-2046	2047-2048	2049-2050	2051-2052	2053-2054	2055-2056	2057-2058	2059-2060	2061-2062	2063-2064	2065-2066	2067-2068	2069-2070	2071-2072	2073-2074	2075-2076	2077-2078	2079-2080	2081-2082	2083-2084	2085-2086	2087-2088	2089-2090	2091-2092	2093-2094	2095-2096	2097-2098	2099-2100	2101-2102	2103-2104	2105-2106	2107-2108	2109-2110	2111-2112	2113-2114	2115-2116	2117-2118	2119-2120	2121-2122	2123-2124	2125-2126	2127-2128	2129-2130	
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Description

The present invention relates to nucleic acids encoding a novel human protein phosphatase of the protein serine/threonine phosphatase family. In particular, it relates to novel DNA sequences encoding a serine/threonine protein phosphatase, to expression plasmids containing said nucleic acids, to host organisms transformed by said expression plasmids, to the production of said protein by culturing said transformant, to antibodies specifically binding to said phosphatase and to agonists and/or antagonists for said protein. Furthermore, the invention relates to serine or threonine residues and epitopes comprising said residues dephosphorylated by said protein and pharmaceutical compositions comprising said protein or agonists or antagonists thereof for the treatment of diseases influenced by changes in phosphorylation which controls e.g. cell proliferation and/or differentiation, to diagnostic kits and to *in vitro* diagnostic methods for the detection of phosphorylation dependent diseases such as e.g. cancer.

Protein phosphorylation-dephosphorylation is a universal mechanism by which different cellular events are regulated. The serine/threonine-specific phosphatases have been classified into four main types according to their *in vitro* specificity for selected substrates and sensitivity to activators and inhibitors (Ingebritsen, T.S. and Cohen, P. (1983) *Eur. J. Biochem.* 182, 255-261). Sequence analysis revealed that they can be classified into two major gene families. The first one includes type 1 (PP1), type 2A (PP2A), and type 2B (PP2B) phosphatases, which share 37 to 59 % sequence identity (Barton, G.J. et al. (1994) *Eur. J. Biochem.* 220, 225-237) in their catalytic domains and are inhibited by okadaic acid (Bialojan, C., and Takai, A. (1988) *Biochem. J.* 256, 283-290). The second family, the Mg^{2+} -dependent phosphatases, also designated type 2C (PP2C), share little sequence similarity with the first family and are insensitive to okadaic acid. cDNA sequences of PP2C α and β from mammalian sources showed > 90 % identity. PP2Cs have been implicated in the regulation of fatty acid and cholesterol biosynthesis (Moore, F. et al. (1991) *Eur. J. Biochem.* 199, 691-697) and heat shock response (Maeda et al. (1993) *Mol. Cell. Biol.* 13, 5408-5417, Shiozaki, K. et al. (1994) *Mol. Cell. Biol.* 14, 3742-375).

The technical problem underlying the present invention is to provide a new human PP2C-like protein phosphatase which is distinct from the other PP2Cs.

The solution to the above technical problem is achieved by providing the embodiments characterized in the claims. Other features and advantages of the invention will be apparent from the description of the preferred embodiments and drawings.

The sequence listings and drawings will now be briefly described.

SEQ ID NO. 1 shows the nucleotide sequence of MP-19, a DNA sequence derived from human placenta.

SEQ ID NO. 2 shows the amino acid sequence of MP-19 as deduced from SEQ ID NO. 1.

SEQ ID NO. 3 shows the nucleotide sequence of MP-19 full-length cDNA derived from human placenta.

SEQ ID NO. 4 shows the amino acid sequence of MP-19 as deduced from SEQ ID NO. 3.

The figures show:

Figure 1 shows the alignment of the amino acid sequence of MP-19 with some related proteins of the PP2C family. The asterisk (*) indicates the position of identical amino acids of the compared amino acid sequences.

MP19-PCR:	amino acid sequence of MP-19 (SEQ ID NO. 2)
PP2C-Human:	human protein phosphatase 2C alpha (Accession No. S87759)
PP2C-Rabbit:	rabbit protein phosphatase 2C alpha (Accession No. S87757)
PP2C-Rat:	rat protein phosphatase 2C (Accession No. J04503)

Figure 2 is a human RNA master blot. The RNA-dot blot analysis shows hybridization of MP-19 PCR probe with different human RNA samples.

A1 whole brain, A2 amygdala, A3 caudate nucleus, A4 cerebellum, A5 cerebral cortex, A6 frontal lobe, A7 hippocampus, A8 medulla oblongata, B1 occipital lobe, B2 putamen, B3 substantia nigra, B4 temporal lobe, B5 thalamus, B6 subthalamic nucleus, B7 spinal cord, B8 blank, C1 heart, C2 aorta, C3 skeletal muscle, C4 colon, C5 bladder, C6 uterus, C7 prostate, C8 stomach, D1 testis, D2 ovary, D3 pancreas, D4 pituitary gland, D5 adrenal gland, D6 thyroid gland, D7 salivary gland, D8 mammary gland, E1 kidney, E2 liver, E3 small intestine, E4 spleen, E5 thymus, E6 peripheral leukocyte, E7 lymph node, E8 bone marrow, F1 appendix, F2 lung, F3 trachea, F4 placenta, F5-F8 blank, G1 fetal brain, G2 fetal heart, G3 fetal kidney, G4 fetal liver, G5 fetal spleen, G6 fetal thymus, G7 fetal lung, G8 blank, H1 yeast total RNA, H2 yeast tRNA, H3 *E. coli* tRNA, H4 *E. coli* DNA, H5 poly (A), H6-H8 human DNA.

Figure 3 shows the detection of MP-19 after IMAc. The Western analysis was performed after purification of MP-19 using Immobilized Metal Ion Affinity Chromatography (IMAC). Positive signals were obtained from fraction 16 to fraction 22.

The amino acid sequence alignment of MP-19 with sequences of different PP2Cs (shown in Figure 1) demonstrates the homology of MP-19 to the PP2C family but implicate also that MP-19 belongs to a new protein phosphatase group. The homology of the derived MP-19 amino acid sequence (aa 1 - aa 226) to PP2C from human, rabbit and rat displays a sequence homology of 21.2 %. Moreover a partial sequence of MP-19 (amino acid sequence 158 - 226) which is in the shown alignment not disrupted by gaps indicates a sequence homology of 39.1 % to the compared PP2Cs.

The present invention relates particularly to a novel serine/threonine protein phosphatase and, preferably, provides DNA sequences contained in the corresponding gene. Such sequences include nucleotide sequences as illustrated in SEQ ID NO. 1 and SEQ ID NO. 3, allelic derivatives of said sequences and DNA sequences degenerated as a result of the genetic code for said sequences. It also includes DNA sequences hybridizing under stringent conditions with the DNA sequence mentioned above. It further includes antisense nucleic acid, preferably antisense MP-19 nucleic acid, directed to the above defined nucleic acid. The terms "nucleic acid sequence" and "nucleotide sequence" refers to DNA or RNA or heterooligomeric sequences, which may be double- or single-stranded.

Although said allelic, degenerate and hybridizing sequences may have structural divergences due to naturally occurring mutations, such as small deletions or substitutions, they will usually still exhibit essentially the same useful properties, allowing their use in basically the same medical or diagnostic applications.

According to the present invention, the term "hybridization" means conventional hybridization conditions, preferably conditions with a salt concentration of 6 x SSC at 62°C to 66°C followed by a one-hour wash with 0.6 x SSC, 0.1% SDS at 62°C to 66°C.

Important biological embodiments of the present invention are DNA sequences of the above and obtainable from vertebrates, preferably mammals such as pig and from rodents such as rat, and in particular from primates such as humans.

Particularly preferred embodiments of the present invention are the DNA sequence termed MP-19 which are shown in SEQ ID NO. 1 and SEQ ID NO. 3. The corresponding transcripts of MP-19 were obtained from human placenta tissue and code for a protein showing considerable amino acid homology to the PP2C proteins (shown in Figure 1 deduced from SEQ ID NO. 1). The protein sequence of rabbit and human PP2C α and rat and rabbit PP2C α are described in Mann et al. (1992) Biochim. Biophys. Acta 1130, 100-104. Some typical sequence homologies, which are specific for the known PP2Cs, were also found in the MP-19 sequence. In the present invention, cloning was carried out according to the method described below. Once the DNA sequence has been cloned, the preparation of host cells capable of producing the PP2C-like protein MP-19 and the production of said protein can be easily accomplished using known recombinant DNA techniques comprising constructing the expression plasmids encoding said protein and transforming a host cell with said expression plasmid, cultivating the transformant in a suitable culture medium, and recovering the product having PP2C-like activity.

Thus, the invention also relates to recombinant molecules comprising DNA sequences as described above, optionally linked to an expression control sequence. Such vectors may be useful in the production of the PP2C-like protein in stable or transiently transformed cells. Several animal, plant, fungal and bacterial systems may be employed for the transformation and subsequent cultivation process. Preferably, expression vectors which can be used in the invention contain sequences necessary for the replication in the host cell and are autonomously replicable. It is also preferable to use vectors containing selectable marker genes which can be easily selected for transformed cells. The necessary operation is well-known to those skilled in the art.

It is another object of the invention to provide a host cell transformed by an expression plasmid of the invention and capable of producing a protein of the serine/threonine phosphatase family. Examples of suitable host cells include various eucaryotic and procaryotic cells, such as *E. coli*, insect cells, plant cells, mammalian cells, and fungi such as yeast.

Another object of the present invention is to provide a PP2C-like protein or a biologically active fragment thereof encoded by the sequences described above and displaying biological features such as dependency of Mg^{2+} (or Mn^{2+}) for activity. Furthermore, the phosphatase catalyzes dephosphorylation of phosphoserine/threonine residues of proteins and peptides phosphorylated by cAMP-dependent protein kinases and protein kinase C. It is insensitive to inhibitors like okadaic acid and calyculin A, heparin and PP1 inhibitors 1 and 2. It does not attack phosphorylase α . It is inhibited by polycations and F^- ions. A preferred substrate for the PP2C-like protein is the SET protein, suggesting capacities possible relevant to therapeutically treatment of leukemia. Furthermore, the PP2C-like protein prefers basic substrates such as histones, and MBP phosphorylated by cAMP-dependent protein kinase, suggesting a special function for this phosphatase in the brain. The amino acid sequences of especially preferred PP2C-like proteins (MP-19) are shown in SEQ ID NO. 2 and SEQ ID NO. 4.

It is a further aspect of the invention to provide a process for the production of PP2C-like proteins. Such a process comprises cultivating a host cell being transformed with a nucleic acid sequence of the present invention in a suitable culture medium and purifying the PP2C-like protein produced. Thus, this process allows the production of the sufficient amount of the desired protein for use in medical treatments. The host cell is obtainable from bacteria such as *Bacillus spec.* or *Escherichia coli*, from fungi such as yeast, from plants such as tobacco, potato, or *Arabidopsis*, and from animals, in particular vertebrate cell lines such as the Mo-, COS- or CHO cell line.

It is another object of the present invention to provide pharmaceutical compositions containing a therapeutically-effective amount of a PP2C-like protein of the present invention and, optionally, a pharmaceutically acceptable carrier and/or diluent, and/or agonists and/or antagonists thereof. Such a therapeutic composition can be used for the treatment of cancer such as leukemia, brain cancer, breast cancer and prostate cancer. The pharmaceutical composition according to the present invention can also be therapeutically applied for degenerative disorders of the CNS, e.g. Alzheimer's disease, Huntington's disease, Parkinson's disease, and epilepsy, and disorders of the reproductive system e.g. fertility disorders or testicular cancer. Another possible clinical application of a PP2C-like protein is the use for treatment of liver diseases, diabetes, and cystic fibrosis. The pharmaceutical composition comprising the protein of the present invention can also be used for treatment of microbial or viral infections. Another application of the pharmaceutical composition of the present invention is the usage of the protein in the regulation of spermatogenesis or the maturation of mammalian germ cells, e.g. for contraception.

Furthermore, the application of the composition is not limited to humans but can include animals, in particular domestic animals, as well.

Finally, another object of the present invention is an antibody or antibody fragment, which is capable of specifically binding to the proteins of the present invention. Methods to raise such specific antibody are known in the art. Such an antibody is preferably a monoclonal antibody. Such antibodies or antibody fragments might be useful for diagnostic methods.

The following examples illustrate in more detail the present invention, but should not be construed as limiting the invention.

Example 1

Isolation of MP-19

For the reverse transcription reaction, 5 µg total RNA (0.5 µg/µl) derived from human placenta tissue was heated for 5 minutes and cooled rapidly on ice for 5 minutes. The reverse transcription reagent mixture containing 5 µg total RNA, 38 u of RNA-guard (Pharmacia), 2.5 µg oligomer d(T)12-18 (Boehringer Mannheim), 5x reaction buffer (250 mM Tris/HCl pH 8.5; 50 mM MgCl₂; 50 mM DTT; 600 mM KCl), 10 mM of each dNTP (Pharmacia), 37.5 u of avian myeloblastosis virus reverse transcriptase (AMV, Boehringer Mannheim). The reaction mixture (20 µl) was incubated for 90 minutes at 42°C. The resulting placenta cDNA pool was stored at -20°C.

For the primary polymerase chain reaction (PCR), a placenta-derived cDNA pool was used as template in a 50 µl reaction mixture. The PCR reaction was carried out in a RoboCycler Gradient 96 (Stratagene). The amplification was performed in 1x PCR-buffer (10 mM Tris/HCl pH 8.3; 50 mM KCl; 0.001 % gelatine), 1 mM of each dNTP (Pharmacia), 100 pmol of each oligonucleotide (ALK6-N2, 5' - TT(CT)(AG)C(AGCT)AT(AGCT)ATAGAAGAAGATGA - 3' and ALK6-R2, 5' - CC(AGCT)CGCCA(CT)TT(AGCT)CCCATCCA - 3') and 1.5 u Taq polymerase (Perkin Elmer). The PCR reaction contained cDNA corresponding to 30 ng of total RNA as starting material. The reaction mixture was overlaid by 40 µl paraffin incubated for 180s/94°C and subjected to 30 cycles (50s/94°C, 90s/48 °C, 60s/72 °C) with an additional extension for 480s/72 °C in the Thermocycler.

A second round of amplification was performed as described above with exception that 5 µl from the first PCR reaction was used as template DNA for the PCR. A 10 µl sample from the second PCR amplification was fractionated by electrophoresis using a 2 % agarose gel in TBE buffer. After electrophoresis amplified DNA corresponding to a molecular weight of about 600-800 bp was excised from the gel and isolated by 3x freeze/thaw cycles (-20 °C / +37 °C) and using the DNA Purification Kit "Easy Pure" (Biozyme, Cat. no. 39001) following the instructions of the manufacturer.

The eluted DNA was amplified a third time as described in the primary PCR with exception that 3 µl of the eluted DNA, resulted from the second round of amplification, was used as template for PCR and the annealing temperature was 56 °C instead of 48 °C. After electrophoresis using a 2 % agarose gel in TBE buffer, a distinct DNA band that corresponds to a molecular weight of about 700 bp was eluted with the extraction method described before. After than an additionally purification using the QIAquick 8 PCR Purification Kit (Qiagen, Cat. no. 28144) following the instructions of the manufacturer, was carried out.

Cloning of the purified DNA was established using the Original TA Cloning Kit (Invitrogen, Cat. no. K2000-40). Plasmid DNA from positive clones was isolated with the QIAwell 8 Plus Plasmid Kit (Qiagen, Cat. no. 16142) and sequenced with an automatic DNA sequencer (ALFexpress, Pharmacia). The resulting DNA sequence was analyzed by a homol-

ogy search with the blast program.

Example 2

5 Isolation of MP-19 full-length cDNA

Isolation of a full-length cDNA clone of MP-19 was performed with a commercial available Human Placenta Lambda cDNA Library (Stratagene, Cat. no. 937225). For screening, a labeled PCR probe was generated from MP-19 DNA (SEQ ID NO. 1). The amplification was performed in 1x PCR-buffer (Qiagen, Germany), 1 mM of dATP, 1 mM of dCTP, 1 mM of dGTP, 0.6 mM of dTTP (Pharmacia, Germany), 0.4 mM of Digoxigenin-11-dUTP (Boehringer, Mannheim), 100 pmol of each oligonucleotide PL19-N1 (5'-GGGCAGAACTGTACACAGGG-3') and PL19-R1 (5'-CATCCAT-GGTGACCTTGCCACC-3') and 1 u Taq DNA-polymerase (Qiagen). The PCR mix was overlaid by 40 µl paraffin, incubated for 180s/94 °C and subjected to 30 cycles (60s/94 °C, 60s/58 °C, 60s/72 °C) with an additional extension for 180s/72 °C.

Prehybridization of plaque lift filters from cDNA library were done at 58 °C for 4 h in 0.25 M Na₂HPO₄, 7 % SDS, 1 % BSA, 1 mM EDTA, pH 7.2. Hybridization was carried out with 50 ng labeled MP19 PCR probe for 15 h under same buffer conditions as prehybridization was done. Filter washed 3 times (5 min, 10 min and 15 min) with 30 mM Na₂HPO₄, 0.1 % SDS at 60 °C. Detection of signals were performed with DIG Luminescent detection kit from Boehringer, Mannheim (Cat. no. 1383514). Proceed from a positive signal, clone 39-1 was isolated and sequenced. The resulting DNA-sequence from clone 39-1 conform to Bp. 387-1641 in SEQ ID NO. 3. To generate the 5' cDNA end of MP-19 full-length cDNA, 1 µg of a Lambda-DNA preparation from Human Placenta Lambda cDNA Library was subjected to PCR. The amplification was performed in 1x PCR-buffer (Qiagen), 1 mM of each dNTP (Pharmacia), 100 pmol of each oligonucleotide MP19-E5 (5'-GGATCCATGGGTGCCTACCTCTCCAGGCC-3') which was derived from an EST sequence (accession no. A115688) and MP19-3 (5'-GCCTGTGTAGGCTTGGCTGTGGGCC-3') and 1 u Taq DNA-polymerase (Qiagen). The reaction was overlaid by 40 µl paraffin incubated for 180s/94 °C and subjected to 30 cycles (60s/94 °C, 60s/74 °C, 60s/72 °C) with an additional extension for 300s/72 °C. The resulting PCR fragment was subcloned in vector pCR 2.1 (Clontech, Germany). Corresponding DNA was digested with restriction endonucleases Bam HI and Stu I. After that MP-19 Bam HI/Stu I fragment was inserted into clone 39-1. The resulted clone was named 28-9 which DNA-sequence present the full-length cDNA-sequence of MP-19 as shown in SEQ ID NO. 3. The corresponding amino acid sequence of MP-19 is shown in SEQ ID NO. 4.

Gene expression of MP-19 in human tissues

Relative expression of the MP-19 gene was determined by Northern blot analyses. A commercial available Human RNA Master Blot (Clontech, Germany, Cat. no. 7770-1, Lot no. 7090716) was hybridized with the digoxigenin labeled MP-19 PCR probe as described in: Isolation of MP-19 full-length cDNA. 50 different human tissues samples were investigated for MP-19 gene expression. Additionally 8 different negative controls from *E. coli*, yeast and human genomic DNA were applied.

Main expression of MP-19 was detected in human testis which is shown in figure 2. Low expression of MP-19 was detected in human pituitary gland, thymus, small intestine and fetal liver. Basal expression of MP-19 was found in all other human RNA samples. No hybridization signals were detected in negative controls.

Expression of MP-19 cDNA in *E. coli*

The cDNA of clone 28-9 was subcloned into the expression plasmid pQE-16 (Qiagen, Germany). This cloning strategy constituted an additional tag of 6 histidine residues at the C-terminus of MP-19. pQE-16 was digested with Bam HI and Bgl II. The 5' part of MP-19 was excised from clone 28-9 with Bam HI and Sac I. To constitute a compatible 3' end of MP-19 for cloning into plasmid pQE-16, a PCR was performed with primer MP19N-Sac I (5'-ACAGCAAGAGTC-CAGCCAGAG-3') and MP19R-Bgl II (5'-AGATCTGTCTCGCTTGGCCTTCTTCTC-3') and template DNA of clone 28-9. 5' and 3' end of MP-19 DNA was ligated into pQE-16 to establish MP-19 with His-tag, which was expressed in *E. coli* strain M15 (Qiagen). For recombinant expression of MP-19, cells were grown in a 5 l fermenter (Bio Console ADI 1035, Applikon, Netherlands) at 37 °C in LB-Medium until an OD₆₀₀ of 2.5 was reached. After induction with 1 mM β-D-thiogalactopyranoside, cells were grown for additional 4 h until OD₆₀₀ of 9.7 was reached. Cells were harvested by centrifugation at 10,000 x g for 30 min, washed once in 500 ml 1 x PBS buffer (30 min at 10,000 x g) and were frozen in aliquots at -80 °C. For preparation of MP-19 protein, 10 g cells were lysed in 100 ml lysis buffer (50 mM NaH₂PO₄, pH 8.0, 300 mM NaCl, 10 mM imidazole, 100 mg lysozyme (Serva, Germany) and 50 u Benzonase (Merck, Germany)) following by sonication 3 times with a ultrasonic processor (UP-200S, Dr. Hielscher GmbH, Germany) for 3 min at 5 kWsec⁻¹ in an ice/water bath. Cell debris was removed by centrifugation for 30 min at 4 °C and 25,000 x g.

Purification of recombinant MP-19

Recombinant expressed MP-19 was purified using Immobilized Metal Ion Affinity Chromatography (IMAC) and Reverse Phase Liquid Chromatography (RPLC). The chromatographic purification was realized using the AKTA-Explorer system (Pharmacia Biotech, Germany). For IMAC, a 1 ml Hi-Trap chelating column (Pharmacia Biotech) is used. Hi-Trap column was activated with 5 column volumes (cv) of 100 mM NiSO_4 , afterwards the column washed with 5 cv water to remove unbound Ni^{2+} . Column equilibration was performed with 5 cv of lysis buffer (50 mM NaH_2PO_4 , 300 mM NaCl, 10 mM Imidazole, pH 8.0). Cell lysat results from 1 g *E. coli* cells was loaded onto column. Afterwards the column washed with lysis buffer to remove unbound protein. Protein were eluted using the following gradient program:

Step 1: 20 mM imidazole to 300 mM imidazole within 20 minutes, step 2: 300 mM imidazol to 500 mM imidazol within 10 minutes using buffer A (50 mM Tris, 300 mM NaCl, 20 mM Imidazole, pH 6.0) and buffer B (50 mM Tris, 300 mM NaCl, 500 mM Imidazole, pH 6.0). Flow rate of chromatography were 1 ml/min, detection were performed at 280 nm. Fractions were analyzed by immunological detection, shown in Figure 3. For further purification, positive fractions containing MP-19 were pooled and loaded onto a Resource RPC (3ml) column (Pharmacia Biotech). Column was equilibrated with buffer A (0.1 % trifluor acetic acid) and protein eluted with a linear gradient of buffer B (0.1 % TFA-90 % acetonitrile). Flow rate of chromatography were 3 ml/min, detection were performed at 215 nm.

Immunological Detection of MP-19

Immunological detection of recombinant MP-19 was performed by western blotting using a commercial available monoclonal mouse antibody against histidine-tag (Dianova, Germany, Cat. no. dia900) in combination with Western Light chemoluminescent detection system using the goat anti-mouse-AP antibody (Tropix, U.S.A.).

Activity assay for recombinant MP-19

A Serine/Threonine Phosphatase Assay System (Promega, Germany, Cat. no. V2460) was used to determine enzymatic activity of recombinant MP-19. This assay use a chemically synthesized phosphopeptide RRA(pT)VA which is a functional substrate for MP-19 phosphatase. The amount of free phosphate which is generated by MP-19 enzymatic reaction was measured by the absorbency of a molybdate-malachite green-phosphate complex (Ekman P. and Jager O. (1993), Anal. Biochem. 214, 138-141, Deana A. D. et al. (1990), Biochimica et Biophysica Acta 1051, 199-202). Assays were performed as described by the manufacturer in PPTase-2C buffer (50 mM imidazole, pH 7.2, 0.2 mM EGTA, 5 mM MgCl_2 , 0.02 % β -mercaptoethanol, 0.1 mg/ml BSA). To determine background of this assay, clone pQE-16-dhfr (Qiagen, Germany) was used, which is identical to pQE-MP-19 with exception that vector pQE-16 inserted a mouse dhfr gene instead of MP-19 phosphatase gene.

Results of activity assay of MP-19 shown in table 1. MP-19 has a significant activity in a MgCl_2 containing buffer, but no activity in a CaCl_2 containing buffer, which shows the requirement to Mg^{2+} . Inhibitors like okadaic acid (10 μM) shows no significant reduction of MP-19 activity. Control expression of the mouse dhfr gene shows no activity in the Serine/Threonine Phosphatase Assay System.

	A	B	C	D (Average A-C)
1	0.000	0.006	-0.006	0.000
2	0.024	0.029	0.023	0.025
3	0.304	0.291	0.298	0.298
4	0.612	0.594	0.597	0.601
5	1.080	1.140	1.137	1.119
6	0.021	0.006	0.018	0.015
7	0.025	0.043	0.018	0.029
8	0.038	0.020	0.018	0.025
9	0.030	0.012	0.013	0.018
10	0.151	0.108	0.174	0.144
11	0.146	0.147	0.139	0.144

Table 1: Activity test of MP-19

- A1-D1: Phosphate standard 0 pmol
 A2-D2: Phosphate standard 100 pmol
 A3-D3: Phosphate standard 500 pmol
 A4-D4: Phosphate standard 1000 pmol
 A5-D5: Phosphate standard 2000 pmol
 A6-D6: mouse dhfr gene with substrate (negative control)
 A7-D7: mouse dhfr gene without substrate (negative control)
 A8-D8: MP-19 with modified PPTase-2C buffer (5mM $MgCl_2$ is replaced by 5 mM $CaCl_2$) and substrate
 A9-D9: MP-19 with PPTase-2C buffer without substrate
 A10-D10: MP-19 with PPTase-2C buffer and substrate
 A11-D11: MP-19 with PPTase-2C buffer, substrate and 10 μ M okadaic acid

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Biopharm GmbH
- (B) STREET: Czernyring 22
- (C) CITY: Heidelberg
- (D) COUNTRY: Germany
- (E) POSTAL CODE (ZIP): 69115

(ii) TITLE OF INVENTION: Nucleic acid encoding a novel human protein phosphatase

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: EP 98107346.3

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 678 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TACGGGCAGA ACTGTCAAA GGGCCCTCCC CACAGCAAAT CTGGAGGTGG GACAGGCGAG 60
 GAACCAAGGT CCCAGGSCCT CAATGGGGAG GCAGGACCTG AGGACTCAAC TAGGGAAACT 120

5 CCTTCACAAG AAAATGGCCC CACAGCCAAG GCCTACACAG GCTTTTCCTC CAACTCGGAA 180
 CGTGGGACTG AAGCAGGCCA AGTTGGTGAG CCTGGCATTG CCACTGGTGA GGCTGGGCCT 240
 TCCTGCTCTT CAGCCTCTGA CAAGCTGCCT CGAGTTGCTA AGTCCAAGTT CTITGAGGAC 300
 AGTGAGGATG AGTCAGATGA GGCAGGAGAA GAAGAGGAAG ACAGTGAGGA ATGCAGCGAG 360
 GAAGAGGATG GCTACAGCAG TGAGGAGCCA GAGAATGAGG AAGATGAGGA TGACACCGAG 420
 10 GAGGCTGAAG AGGACGATGA AGAAGAAGAA GAAGAGATGA TGGTGCCAGG GATGGAAGGC 480
 AAAGAGGAGC CTGGCTCTGA CAGTGGTACA ACAGCGGTGG TGGCCCTGAT ACGAGGGAAG 540
 CAGTTGATTG TAGCCAACGC AGGAGACTCT CGCTGTGTGG TATCTGAGGC TGGCAAMGCT 600
 15 TTAGACATGT CCTATGATCA CAAACCAGAG GATGAAGTAG AACTAGCACG CATCAAGAAT 660
 GCTGGTGGCA AGTCAACC 678

(2) INFORMATION FOR SEQ ID NO: 2:

- 20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 226 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 25 (ii) MOLECULE TYPE: peptide
 (iii) HYPOTHETICAL: NO
 (iv) ANTI-SENSE: NO
 30 (vi) ORIGINAL SOURCE:
 (F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

35 Tyr Gly Gln Asn Cys His Lys Gly Pro Pro His Ser Lys Ser Gly Gly
 1 5 10
 40 Gly Thr Gly Glu Glu Pro Gly Ser Gln Gly Leu Asn Gly Glu Ala Gly
 20 25 30
 Pro Glu Asp Ser Thr Arg Glu Thr Pro Ser Gln Glu Asn Gly Pro Thr
 35 40 45
 45 Ala Lys Ala Tyr Thr Gly Phe Ser Ser Asn Ser Glu Arg Gly Thr Glu

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	50	55	60
5	Ala Gly Gln Val Gly Glu Pro Gly Ile Pro Thr Gly Glu Ala Gly Pro 65 70 75 80		
	Ser Cys Ser Ser Ala Ser Asp Lys Leu Pro Arg Val Ala Lys Ser Lys 85 90 95		
10	Phe Phe Glu Asp Ser Glu Asp Glu Ser Asp Glu Ala Glu Glu Glu 100 105 110		
	Glu Asp Ser Glu Glu Cys Ser Glu Glu Glu Asp Gly Tyr Ser Ser Glu 115 120 125		
15	Glu Ala Glu Asn Glu Glu Asp Glu Asp Thr Glu Glu Ala Glu Glu 130 135 140		
	Asp Asp Glu Glu Glu Glu Glu Glu Met Met Val Pro Gly Met Glu Gly 145 150 155 160		
20	Lys Glu Glu Pro Gly Ser Asp Ser Gly Thr Thr Ala Val Val Ala Leu 165 170 175		
	Ile Arg Gly Lys Gln Leu Ile Val Ala Asn Ala Gly Asp Ser Arg Cys 180 185 190		
25	Val Val Ser Glu Ala Gly Lys Ala Leu Asp Met Ser Tyr Asp His Lys 195 200 205		
	Pro Glu Asp Glu Val Glu Leu Ala Arg Ile Lys Asn Ala Gly Gly Lys 210 215 220		
30	Val Thr 225		

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1641 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (F) TISSUE TYPE: human placenta

EP 0 874 052 A2

(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

	ATGGGTGCTT ACCTCTCCCA GCCCAACACG GTGAAGTGCT CCGGGGACGG GGTCGGCGCC	60
5	CCGCGCCTGC CGCTGCCCTA CGGCTTCTCC GCCATGCAAG GCTGGCGCGT CTCCATTGGAG	120
	GATGCTCACA ACTGTATTCC TGAGCTGGAC AGTGAGACAG CCATGTTTTT TGTCTACGAT	180
	GGACATGGAG GGGAGGAAGT TGCTTTGTAC TGTGCCAAAT ATCTTCTCTA TATCATCAAA	240
10	GATCAGAAGG CCTACAAGGA AGGCAAGCTA CAGAAGGCTT TAGAAGATGC TTCTTTGGCT	300
	ATTGACGCCA AATTGACCAC TGAAGAAGTC ATTAAGAGC TGGCACAGAT TGCAGGGCGA	360
	CCCACTGAGG ATGAAGATGA AAAAGAAAAA TAGCTGATG AAGATGATGT GGACAATGAG	420
15	GAGGCTGCAC TGCTGCATGA AGAGGCTACC ATGACTATTG AAGAGCTGCT GACACGCTAC	480
	GGGCAGAACT GTCACAAGGG CCTTCCCCAC AGCAAACTCG GAGGTGGGAC AGGCGAGGAA	540
	CCAGGGTCCC AGGGCCTCAA TGGGGAGGCA GGACCTGAGG ACTCAACTAG GGAAGCTCCT	600
20	TCACAAGAAA ATGGCCCCAC AGCCAAGGCC TACACAGGCT TTTCTCCAA CTGCGAACGT	660
	GGGACTGAGG CAGGCCAAGT TGTGAGCCTT GGCATTCCCA CTGGTGAGGC TGGGCTTCC	720
	TGCTCTTCAG CCTCTGACAA GCTGCTCTGA GTTGCTAAGT CCAAGTTCTT TGAGGACAGT	780
25	GAGGATGAGT CAGATGAGGC GGAGGAAGAA GAGGAAGACA GTGAGGAATG CAGCGAGGAA	840
	GAGGATGGCT ACAGCAGTGA GGAGGCAGAG AATGAGGAAG ATGAGGATGA CACCGAGGAG	900
	GCTGAAGAGG ACGATGAAGA AGAAGAAGAA GAGATGATGG TGCCAGGGAT GGAAGGCAAA	960
30	GAGGAGCCTG GCTCTGACAG TGGTACAACA CGGTGGTGG CCCTGATACC AGGGAAGCAG	1020
	TTGATTGTAG CCAACGCAGG AGACTCTCGC TGTGTGGTAT CTGAGGCTGG CAAAGCTTTA	1080
	GACATGTCCT ATGATCACAA ACCAGAGGAT GAAGTAGAAC TAGCACGCT CAAGAATGCT	1140
35	GGTGGCAAGG TCACCATGGA TGGGCGAGTC AACGGGGGCC TCAACCTCTC CAGAGCCATT	1200
	GGGGACCATT TCTATAAGAG AAACAAGAAC CTGCCACCTG AGGAACAGAT GATTTCAGCC	1260
	CTTCTTGACA TCAAGGTGCT GACTCTCACT GACGACCATG AATTATGGT CATTGCCTGT	1320
40	GATGGCATCT GGAATGTGAT GAGCAGCCAG GAAGTTGTAG ATTTCAITTA ATCAAAGATC	1380
45		
50		
55		

AGCCAGCGTG ATGAAATG GAGCTTCGG TTATTGTCAT CCAITGTGGA AGAGCTGCTG 1440
 GATCAGTGCC TGGCACCAGA CACTTCTGGG GATGGTACAG GGTGTGACAA CATGACCTGC 1500
 5 ATCATCATTT GCTTCAAGCC CCGAAACACA GCAGAGCTCC AGCCAGAGAG TGGCAAGCGA 1560
 AACTAGAGG AGTGCTCTC TACTGAGGG GCTGAAGAAA ATGGCAACAG CGACAAGAAG 1620
 AAGAAGGCCA AGCGAGACTA G 1641

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 546 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (P) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Gly Ala Tyr Leu Ser Gln Pro Asn Thr Val Lys Cys Ser Gly Asp
 1 5 10 15
 Gly Val Gly Ala Pro Arg Leu Pro Leu Pro Tyr Gly Phe Ser Ala Met
 20 25 30
 Gln Gly Trp Arg Val Ser Met Glu Asp Ala His Asn Cys Ile Pro Glu
 35 40 45
 Leu Asp Ser Glu Thr Ala Met Phe Ser Val Tyr Asp Gly His Gly Gly
 50 55 60
 Glu Glu Val Ala Leu Tyr Cys Ala Lys Tyr Leu Pro Asp Ile Ile Lys
 65 70 75 80
 Asp Gln Lys Ala Tyr Lys Glu Gly Lys Leu Gln Lys Ala Leu Glu Asp
 85 90 95
 Ala Phe Leu Ala Ile Asp Ala Lys Leu Thr Thr Glu Glu Val Ile Lys
 100 105 110

Claims

1. A nucleic acid comprising a nucleotide sequence encoding a human serine/threonine phosphatase or a functional fragment thereof that is capable of dephosphorylating serine or threonine residues, wherein the nucleotide sequence comprises:

(a) the nucleotides as shown in SEQ ID NO. 1 or SEQ ID NO. 3; or

(b) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequence of (a); or

(c) an allelic derivative of the sequences of (a) or (b); or

(d) a DNA sequence which is capable of hybridizing to the sequences in (a), (b) and (c), and encoding a protein containing the amino acid sequence as depicted in SEQ ID NO. 2 or SEQ ID NO. 4; or

(e) a nucleotide sequence which is capable of hybridizing to the DNA sequences in (a), (b), (c) and (d), and encoding a protein having essentially the same biological properties as the protein defined in (d).

2. The nucleic acid according to claim 1, wherein the nucleotide sequence is a vertebrate DNA sequence, a mammalian sequence, preferably a primate, human, porcine, or rodent, preferably a rat or rabbit, DNA sequence
3. A recombinant molecule comprising a nucleic acid according to claim 1 or 2.
4. The recombinant molecule according to claim 3, wherein said nucleic acid sequence is functionally linked to an expression-control sequence.
5. A host containing the nucleic acid according to claim 1 or 2, or the recombinant molecule according to claim 3 or 4.
6. The host according to claim 5, which is a bacterium, a fungus, a plant cell, an animal or a human cell.
7. A process for the production of a PP2C-like protein comprising cultivating a host according to claim 5 or 6 and recovering said PP2C-like protein from the culture
8. A PP2C-like protein or a biologically active fragment thereof encoded by a nucleic acid according to claim 1 or 2 or by a recombinant molecule according to claim 3 or 4.
9. The protein according to claim 8, comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4.
10. An agonist as a substitute for the protein of claim 8 or 9.
11. An antagonist directed to the protein of claim 8 or 9.
12. A pharmaceutical composition containing the protein or a biologically active fragment thereof according to claim 8 and 9 or the agonist according to claim 10 or the antagonist according to claim 11, and optionally, a pharmaceutically acceptable carrier and/or diluent.
13. The pharmaceutical composition according to claim 12 for the treatment of leukemia, brain cancer, breast cancer, prostate cancer, Alzheimer's disease, Huntington's disease, Parkinson's disease, and epilepsy, and of disorders of the reproductive system, or for the regulation of spermatogenesis or the maturation of mammalian germ cells.
14. An antibody or antibody fragment which is capable of specifically binding to the protein of claim 8 and 9 or to the agonist of claim 10 or to the antagonist of claim 11.
15. The antibody according to claim 14, which is a monoclonal antibody.
16. Use of an antibody or antibody fragment according to claim 14 or 15 for detecting the protein or a biologically active fragment thereof as defined in claim 8 or 9.
17. A diagnostic kit containing the agonist according to claim 10 or the antagonist according to claim 11 or the antibody or antibody fragment according to claim 14 or 15.

Fig. 1

MP19-PCR	YGQNCNKGPP	HSKSGGGTGE	EPGSQGLNGS	AGPEDSTRET	PSQENPTAK	50
PP2C-Human	MGAFLDKPKM	EKHNAQQGG	----NGLAYG	LSSNQGWRE	MEDANTAVIG	45
PP2C-Rabbit	MGAFLDKPKM	EKHNAQQGG	----NGLRYG	LSSNQGWRE	MEDANTAVIG	45
PP2C-Rat	MGAFLDKPKM	EKHNAQQGG	----NGLRYG	LSSNQGWRE	MEDANTAVIG	45
	*	*	*	*	*	
MP19-PCR	AYTGFSNSSE	RGTEAGQVGE	PGIPTGEAGP	SCSSASDKLP	RVAKSKFFED	100
PP2C-Human	LP5GLESWSF	FAVYDGHAG	----SQVAK	YCC--EHLLD	HITNQGDFKG	87
PP2C-Rabbit	LP5GLETWSF	FAVYDGHAG	----SQVAK	YCC--EHLLD	HITNQGDFKG	87
PP2C-Rat	LP5GLETWSF	FAVYDGHAG	----SQVAK	YCC--EHLLD	HITNQGDFKG	87
	*	*	*	*	*	
MP19-PCR	SEDESDEAEE	EEDSEECSE	EEDGYSSEEA	ENEEEDDOTE	EAFEDDEEEE	150
PP2C-Human	SAGAP-SVEN	VKNGI-----	-RTGF-----	-----LEID		109
PP2C-Rabbit	SAGAP-SVEN	VKNGI-----	-RTGF-----	-----LEID		109
PP2C-Rat	SAGAP-SVEN	VKNGI-----	-RTGF-----	-----LEID		109
	*	*	*	*	*	
MP19-PCR	EEMMVPGMG	KEEFGSDSGT	TAVVALIRGK	QLIVANAGDS	RCVVSSEACKA	200
PP2C-Human	EHMV--MSE	KKHGADRSGS	TAVGVLSIPQ	HTYFINGGDS	RGLLCRNKRV	157
PP2C-Rabbit	EHMV--MSE	KKHGADRSGS	TAVGVLSIPQ	HTYFINGGDS	RGLLCRNKRV	157
PP2C-Rat	EHMV--MSE	KKHGADRSGS	TAVGVLSIPQ	HTYFINGGDS	RGLLCRNKRV	157
	*	*	*	*	*	
	*	*	*	*	*	
MP19-PCR	LDMSYDHKPE	DEVELARIKN	AGGKVT			226
PP2C-Human	HFFTQDNKPS	NPLEKERIQN	AGGSVM			183
PP2C-Rabbit	HFFTQDNKPS	NPLEKERIQN	AGGSVM			183
PP2C-Rat	HFFTQDNKPS	NPLEKERIQN	AGGSVM			183
	****	*	**	****	*	

Figure 2

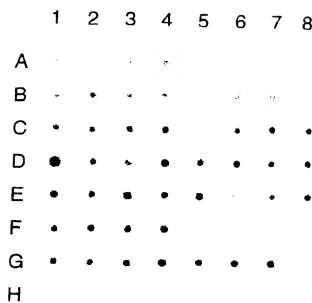
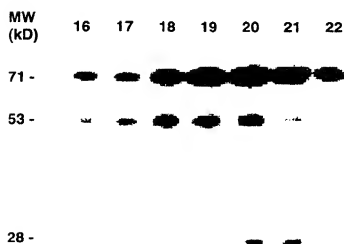
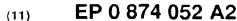


Figure 3





(12)

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(54) **Nucleic acid encoding a human protein phosphatase**

(57) The present invention relates to nucleic acids encoding a novel human protein phosphatase of the family of protein serine/threonine phosphatases. In particular, it relates to novel DNA sequences encoding serine/threonine protein phosphatase, to expression plasmids containing said nucleic acids, to host organisms transformed by said expression plasmids, to the production of said protein by culturing said transformant, to antibodies specifically binding to said phosphatase and to agonists and/or antagonists for said protein, and to antisense MP-19 nucleic acid. Furthermore, the invention relates to serine or threonine residues and epitopes comprising said residues dephosphorylated by said protein and pharmaceutical compositions comprising said protein or agonists or antagonists thereof for the treatment of diseases influenced by changes in phosphorylation which controls e.g. cell proliferation and/or differentiation, to diagnostic kits and to *in vitro* diagnostic methods for the detection of phosphorylation dependent diseases such as e.g. cancer.

59 1

[illegible]

Description

The present invention relates to nucleic acids encoding a novel human protein phosphatase of the protein serine/threonine phosphatase family. In particular, it relates to novel DNA sequences encoding a serine/threonine protein phosphatase, to expression plasmids containing said nucleic acids, to host organisms transformed by said expression plasmids, to the production of said protein by culturing said transformant, to antibodies specifically binding to said phosphatase and to agonists and/or antagonists for said protein. Furthermore, the invention relates to serine or threonine residues and epitopes comprising said residues dephosphorylated by said protein and pharmaceutical compositions comprising said protein or agonists or antagonists thereof for the treatment of diseases influenced by changes in phosphorylation which controls e.g. cell proliferation and/or differentiation, to diagnostic kits and to *in vitro* diagnostic methods for the detection of phosphorylation dependent diseases such as e.g. cancer.

Protein phosphorylation-dephosphorylation is a universal mechanism by which different cellular events are regulated. The serine/threonine-specific phosphatases have been classified into four main types according to their *in vitro* specificity for selected substrates and sensitivity to activators and inhibitors (Ingebritsen, T.S. and Cohen, P. (1983) *Eur. J. Biochem.* 182, 255-261). Sequence analysis revealed that they can be classified into two major gene families. The first one includes type 1 (PP1), type 2A (PP2A), and type 2B (PP2B) phosphatases, which share 37 to 59 % sequence identity (Barton, G.J. et al., (1994) *Eur. J. Biochem.* 220, 225-237) in their catalytic domains and are inhibited by okadaic acid (Bialojan, C., and Takai, A. (1988) *Biochem. J.* 256, 283-290). The second family, the Mg^{2+} -dependent phosphatases, also designated type 2C (PP2C), share little sequence similarity with the first family and are insensitive to okadaic acid. cDNA sequences of PP2C α and β from mammalian sources showed > 90 % identity. PP2Cs have been implicated in the regulation of fatty acid and cholesterol biosynthesis (Moore, F. et al. (1991) *Eur. J. Biochem.* 199, 691-697) and heat shock response (Maeda et al. (1993) *Mol. Cell. Biol.* 13, 5408-5417; Shiozaki, K. et al. (1994) *Mol. Cell. Biol.* 14, 3742-375).

The technical problem underlying the present invention is to provide a new human PP2C-like protein phosphatase which is distinct from the other PP2Cs.

The solution to the above technical problem is achieved by providing the embodiments characterized in the claims. Other features and advantages of the invention will be apparent from the description of the preferred embodiments and drawings.

The sequence listings and drawings will now be briefly described.

SEQ ID NO. 1 shows the nucleotide sequence of MP-19, a DNA sequence derived from human placenta.

SEQ ID NO. 2 shows the amino acid sequence of MP-19 as deduced from SEQ ID NO. 1.

SEQ ID NO. 3 shows the nucleotide sequence of MP-19 full-length cDNA derived from human placenta.

SEQ ID NO. 4 shows the amino acid sequence of MP-19 as deduced from SEQ ID NO. 3.

The figures show:

Figure 1 shows the alignment of the amino acid sequence of MP-19 with some related proteins of the PP2C family. The asterisk (*) indicates the position of identical amino acids of the compared amino acid sequences.

MP19-PCR: amino acid sequence of MP-19 (SEQ ID NO. 2)
 PP2C-Human: human protein phosphatase 2C alpha (Accession No. S87759)
 PP2C-Rabbit: rabbit protein phosphatase 2C alpha (Accession No. S87757)
 PP2C-Rat: rat protein phosphatase 2C (Accession No. J04503)

Figure 2 is a human RNA master blot. The RNA-dot blot analysis shows hybridization of MP-19 PCR probe with different human RNA samples.

A1 whole brain, A2 amygdala, A3 caudate nucleus, A4 cerebellum, A5 cerebral cortex, A6 frontal lobe, A7 hippocampus, A8 medulla oblongata, B1 occipital lobe, B2 putamen, B3 substantia nigra, B4 temporal lobe, B5 thalamus, B6 subthalamic nucleus, B7 spinal cord, B8 blank, C1 heart, C2 aorta, C3 skeletal muscle, C4 colon, C5 bladder, C6 uterus, C7 prostate, C8 stomach, D1 testis, D2 ovary, D3 pancreas, D4 pituitary gland, D5 adrenal gland, D6 thyroid gland, D7 salivary gland, D8 mammary gland, E1 kidney, E2 liver, E3 small intestine, E4 spleen, E5 thymus, E6 peripheral leukocyte, E7 lymph node, E8 bone marrow, F1 appendix, F2 lung, F3 trachea, F4 placenta, F5-F8 blank, G1 fetal brain, G2 fetal heart, G3 fetal kidney, G4 fetal liver, G5 fetal spleen, G6 fetal thymus, G7 fetal lung, G8 blank, H1 yeast total RNA, H2 yeast tRNA, H3 *E. coli* rRNA, H4 *E. coli* DNA, H5 poly (A), H6-H8 human DNA.

Figure 3 shows the detection of MP-19 after IMAC. The Western analysis was performed after purification of MP-19 using Immobilized Metal Ion Affinity Chromatography (IMAC). Positive signals were obtained from fraction 16 to fraction 22.

The amino acid sequence alignment of MP-19 with sequences of different PP2Cs (shown in Figure 1) demonstrates the homology of MP-19 to the PP2C family but implicate also that MP-19 belongs to a new protein phosphatase group. The homology of the derived MP-19 amino acid sequence (aa 1 - aa 226) to PP2C from human, rabbit and rat displays a sequence homology of 21.2 %. Moreover a partial sequence of MP-19 (amino acid sequence 158 - 226) which is in the shown alignment not disrupted by gaps indicates a sequence homology of 39.1 % to the compared PP2Cs.

The present invention relates particularly to a novel serine/threonine protein phosphatase and, preferably, provides DNA sequences contained in the corresponding gene.

Such sequences include nucleotide sequences as illustrated in SEQ ID NO. 1 and SEQ ID NO. 3, allelic derivatives of said sequences and DNA sequences degenerated as a result of the genetic code for said sequences. It also includes DNA sequences hybridizing under stringent conditions with the DNA sequence mentioned above. It further includes antisense nucleic acid, preferably antisense MP-19 nucleic acid, directed to the above defined nucleic acid. The terms "nucleic acid sequence" and "nucleotide sequence" refers to DNA or RNA or heterooligomeric sequences, which may be double- or single-stranded.

Although said allelic, degenerate and hybridizing sequences may have structural divergences due to naturally occurring mutations, such as small deletions or substitutions, they will usually still exhibit essentially the same useful properties, allowing their use in basically the same medical or diagnostic applications.

According to the present invention, the term "hybridization" means conventional hybridization conditions, preferably conditions with a salt concentration of 6 x SSC at 62°C to 66°C followed by a one-hour wash with 0.6 x SSC, 0.1% SDS at 62°C to 66°C.

Important biological embodiments of the present invention are DNA sequences of the above and obtainable from vertebrates, preferably mammals such as pig and from rodents such as rat, and in particular from primates such as humans.

Particularly preferred embodiments of the present invention are the DNA sequence termed MP-19 which are shown in SEQ ID NO. 1 and SEQ ID NO. 3. The corresponding transcripts of MP-19 were obtained from human placenta tissue and code for a protein showing considerable amino acid homology to the PP2C proteins (shown in Figure 1 deduced from SEQ ID NO. 1). The protein sequence of rabbit and human PP2C α and rat and rabbit PP2C α are described in Mann et al. (1992) Biochim. Biophys. Acta 1130, 100-104. Some typical sequence homologies, which are specific for the known PP2Cs, were also found in the MP-19 sequence. In the present invention, cloning was carried out according to the method described below. Once the DNA sequence has been cloned, the preparation of host cells capable of producing the PP2C-like protein MP-19 and the production of said protein can be easily accomplished using known recombinant DNA techniques comprising constructing the expression plasmids encoding said protein and transforming a host cell with said expression plasmid, cultivating the transformant in a suitable culture medium, and recovering the product having PP2C-like activity.

Thus, the invention also relates to recombinant molecules comprising DNA sequences as described above, optionally linked to an expression control sequence. Such vectors may be useful in the production of the PP2C-like protein in stable or transiently transformed cells. Several animal, plant, fungal and bacterial systems may be employed for the transformation and subsequent cultivation process. Preferably, expression vectors which can be used in the invention contain sequences necessary for the replication in the host cell and are autonomously replicable. It is also preferable to use vectors containing selectable marker genes which can be easily selected for transformed cells. The necessary operation is well-known to those skilled in the art.

It is another object of the invention to provide a host cell transformed by an expression plasmid of the invention and capable of producing a protein of the serine/threonine phosphatase family. Examples of suitable host cells include various eucaryotic and procaryotic cells, such as *E. coli*, insect cells, plant cells, mammalian cells, and fungi such as yeast.

Another object of the present invention is to provide a PP2C-like protein or a biologically active fragment thereof encoded by the sequences described above and displaying biological features such as dependency of Mg^{2+} (or Mn^{2+}) for activity. Furthermore, the phosphatase catalyzes dephosphorylation of phosphoserine/threonine residues of proteins and peptides phosphorylated by cAMP-dependent protein kinases and protein kinase C. It is insensitive to inhibitors like okadaic acid and calyculin A, heparin and PP1 inhibitors 1 and 2. It does not attack phosphotyrosine α . It is inhibited by polyocations and F^{-} ions. A preferred substrate for the PP2C-like protein is the SET protein, suggesting capacities possible relevant to therapeutically treatment of leukemia. Furthermore, the PP2C-like protein prefers basic substrates such as histones, and MBP phosphorylated by cAMP-dependent protein kinase, suggesting a special function for this phosphatase in the brain. The amino acid sequences of especially preferred PP2C-like proteins (MP-19) are shown in SEQ ID NO. 2 and SEQ ID NO. 4.

It is a further aspect of the invention to provide a process for the production of PP2C-like proteins. Such a process comprises cultivating a host cell being transformed with a nucleic acid sequence of the present invention in a suitable culture medium and purifying the PP2C-like protein produced. Thus, this process allows the production of the sufficient amount of the desired protein for use in medical treatments. The host cell is obtainable from bacteria such as *Bacillus spec.* or *Escherichia coli*, from fungi such as yeast, from plants such as tobacco, potato, or *Arabidopsis*, and from animals, in particular vertebrate cell lines such as the Mo-, COS- or CHO cell line.

It is another object of the present invention to provide pharmaceutical compositions containing a therapeutically-effective amount of a PP2C-like protein of the present invention and, optionally, a pharmaceutically acceptable carrier and/or diluent, and/or agonists and/or antagonists thereof. Such a therapeutic composition can be used for the treatment of cancer such as leukemia, brain cancer, breast cancer and prostate cancer. The pharmaceutical composition according to the present invention can also be therapeutically applied for degenerative disorders of the CNS, e.g. Alzheimer's disease, Huntington's disease, Parkinson's disease, and epilepsy, and disorders of the reproductive system e.g. fertility disorders or testicular cancer. Another possible clinical application of a PP2C-like protein is the use for treatment of liver diseases, diabetes, and cystic fibrosis. The pharmaceutical composition comprising the protein of the present invention can also be used for treatment of microbial or viral infections. Another application of the pharmaceutical composition of the present invention is the usage of the protein in the regulation of spermatogenesis or the maturation of mammalian germ cells, e.g. for contraception.

Furthermore, the application of the composition is not limited to humans but can include animals, in particular domestic animals, as well.

Finally, another object of the present invention is an antibody or antibody fragment, which is capable of specifically binding to the proteins of the present invention. Methods to raise such specific antibody are known in the art. Such an antibody is preferably a monoclonal antibody. Such antibodies or antibody fragments might be useful for diagnostic methods.

The following examples illustrate in more detail the present invention, but should not be construed as limiting the invention.

Example 1

Isolation of MP-19

For the reverse transcription reaction, 5 µg total RNA (0.5 µg/µl) derived from human placenta tissue was heated for 5 minutes and cooled rapidly on ice for 5 minutes. The reverse transcription reagent mixture containing 5 µg total RNA, 38 µl of RNA-guard (Pharmacia), 2.5 µg oligomer d(T)12-18 (Boehringer Mannheim), 5x reaction buffer (250 mM Tris/HCl pH 8.5, 50 mM MgCl₂, 50 mM DTT, 600 mM KCl), 10 mM of each dNTP (Pharmacia), 37.5 µl of avian myeloblastosis virus reverse transcriptase (AMV, Boehringer Mannheim). The reaction mixture (20 µl) was incubated for 90 minutes at 42°C. The resulting placenta cDNA pool was stored at -20°C.

For the primary polymerase chain reaction (PCR), a placenta-derived cDNA pool was used as template in a 50 µl reaction mixture. The PCR reaction was carried out in a RoboCycler Gradient 96 (Stratagene). The amplification was performed in 1x PCR-buffer (10 mM Tris/HCl pH 8.3, 50 mM KCl, 0.001 % gelatine), 1 mM of each dNTP (Pharmacia), 100 pmol of each oligonucleotide (ALK6-N2, 5' - TT(CT)(AG)C(AGCT)AT(AGCT)ATAGAGAAGATGA - 3' and ALK6-R2, 5' - CC(AGCT)CGCCCA(CT)TT(AGCT)CCCATCCA - 3') and 1.5 µl Taq polymerase (Perkin Elmer). The PCR reaction contained cDNA corresponding to 30 ng of total RNA as starting material. The reaction mixture was overlaid by 40 µl paraffin incubated for 180s/94°C and subjected to 30 cycles (50s/94°C, 90s/48 °C, 60s/72 °C) with an additional extension for 480s/72 °C in the Thermocycler.

A second round of amplification was performed as described above with exception that 5 µl from the first PCR reaction was used as template DNA for the PCR. A 10 µl sample from the second PCR amplification was fractionated by electrophoresis using a 2 % agarose gel in TBE buffer. After electrophoresis amplified DNA corresponding to a molecular weight of about 600-800 bp was excised from the gel and isolated by 3x freeze/thaw cycles (-20 °C / + 37 °C) and using the DNA Purification Kit "Easy Pure" (Biozyme, Cat. no. 39001) following the instructions of the manufacturer.

The eluted DNA was amplified a third time as described in the primary PCR with exception that 3 µl of the eluted DNA, resulted from the second round of amplification, was used as template for PCR and the annealing temperature was 56 °C instead of 48 °C. After electrophoresis using a 2 % agarose gel in TBE buffer, a distinct DNA band that corresponds to a molecular weight of about 700 bp was eluted with the extraction method described before. After than an additionally purification using the QIAquick 8 PCR Purification Kit (Qiagen, Cat. no. 28144) following the instructions of the manufacturer, was carried out.

Cloning of the purified DNA was established using the Original TA Cloning Kit (Invitrogen, Cat. no. K2000-40). Plasmid DNA from positive clones was isolated with the QIAwell 8 Plus Plasmid Kit (Qiagen, Cat. no. 16142) and sequenced with an automatic DNA sequencer (ALFexpress, Pharmacia). The resulting DNA sequence was analyzed by a homol-

ogy search with the blast program.

Example 2

5 Isolation of MP-19 full-length cDNA

Isolation of a full-length cDNA clone of MP-19 was performed with a commercial available Human Placenta Lambda cDNA Library (Stratagene, Cat. no. 937225). For screening, a labeled PCR probe was generated from MP-19 DNA (SEQ ID NO. 1). The amplification was performed in 1x PCR-buffer (Qiagen, Germany), 1 mM of dATP, 1 mM of dCTP, 1 mM of dGTP, 0.6 mM of dTTP (Pharmacia, Germany), 0.4 mM of Digoxigenin-11-dUTP (Boehringer, Mannheim), 100 pmol of each oligonucleotide PL19-N1 (5'-GGGCAGAACTGTCAACAAGG-3') and PL19-R1 (5'-CATCCATGGTGACCTTGCCACC-3') and 1 U Taq DNA-polymerase (Qiagen). The PCR mix was overlaid by 40 µl paraffin, incubated for 180s/94 °C and subjected to 30 cycles (60s/94 °C, 60s/58 °C, 60s/72 °C) with an additional extension for 180s/72 °C.

Prehybridization of plaque lift filters from cDNA library were done at 58 °C for 4 h in 0.25 M Na₂HPO₄, 7 % SDS, 1 % BSA, 1 mM EDTA, pH 7.2. Hybridization was carried out with 50 ng labeled MP19 PCR probe for 15 h under same buffer conditions as prehybridization was done. Filter washed 3 times (5 min, 10 min and 15 min) with 30 mM Na₂HPO₄, 0.1 % SDS at 60 °C. Detection of signals were performed with DIG Luminescent detection kit from Boehringer, Mannheim (Cat. no. 1363514). Proceed from a positive signal, clone 39-1 was isolated and sequenced. The resulting DNA-sequence from clone 39-1 conform to Bp. 387-1641 in SEQ ID NO. 3. To generate the 5' cDNA end of MP-19 full-length cDNA, 1 µg of a Lambda-DNA preparation from Human Placenta Lambda cDNA Library was subjected to PCR. The amplification was performed in 1x PCR-buffer (Qiagen), 1 mM of each dNTP (Pharmacia), 100 pmol of each oligonucleotide MP19-E5 (5'-GGATCCATGGGTGCTACCTCTCCAGCCC-3') which was derived from an EST sequence (accession no. AA115688) and MP19-3 (5'-GCCTGTGTAGGCCCTGGCTGGGGCC-3') and 1 U Taq DNA-polymerase (Qiagen). The reaction was overlaid by 40 µl paraffin incubated for 180s/94 °C and subjected to 30 cycles (60s/94 °C, 60s/74 °C, 60s/72 °C) with an additional extension for 300s/72 °C. The resulting PCR fragment was subcloned in vector pCR 2.1 (Clontech, Germany). Corresponding DNA was digested with restriction endonucleases Bam HI and Stu I. After that MP-19 Bam HI/Stu I fragment was inserted into clone 39-1. The resulted clone was named 28-9 which DNA-sequence present the full-length cDNA-sequence of MP-19 as shown in SEQ ID NO. 3. The corresponding amino acid sequence of MP-19 is shown in SEQ ID NO. 4.

Gene expression of MP-19 in human tissues

Relative expression of the MP-19 gene was determined by Northern blot analyses. A commercial available Human RNA Master Blot (Clontech, Germany, Cat. no. 7770-1, Lot no. 7090716) was hybridized with the digoxigenin labeled MP-19 PCR probe as described in: Isolation of MP-19 full-length cDNA. 50 different human tissues samples were investigated for MP-19 gene expression. Additionally 8 different negative controls from *E. coli*, yeast and human genomic DNA were applied.

Main expression of MP-19 was detected in human testis which is shown in figure 2. Lower expression of MP-19 was detected in human pituitary gland, thymus, small intestine and fetal liver. Basal expression of MP-19 was found in all other human RNA samples. No hybridization signals were detected in negative controls.

Expression of MP-19 cDNA in *E. coli*

The cDNA of clone 28-9 was subcloned into the expression plasmid pQE-16 (Qiagen, Germany). This cloning strategy constituted an additional tag of 6 histidine residues at the C-terminus of MP-19. pQE-16 was digested with Bam HI and Bgl II. The 5' part of MP-19 was excised from clone 28-9 with Bam HI and Sac I. To constitute a compatible 3' end of MP-19 for cloning into plasmid pQE-16, a PCR was performed with primer MP19N-Sac I (5'-ACAGCAGAGCTC-CAGCCAGAG-3') and MP19R-Bgl II (5'-AGATCTGTCTCGCTTGCCCTCTTCTTC-3') and template DNA of clone 28-9. 5' and 3' end of MP-19 DNA was ligated into pQE-16 to establish MP-19 with His-tag, which was expressed in *E. coli* strain M15 (Qiagen). For recombinant expression of MP-19, cells were grown in a 5 l fermenter (Bio Console ADI 1035, Applikon, Netherlands) at 37 °C in LB-Medium until an OD₆₀₀ of 2.5 was reached. After induction with 1 mM β-D-thiogalactopyranoside, cells were grown for additional 4 h until OD₆₀₀ of 9.7 was reached. Cells were harvested by centrifugation at 10,000 x g for 30 min, washed once in 500 ml 1 x PBS buffer (30 mM at 10,000 x g) and were frozen in aliquots at -80 °C. For preparation of MP-19 protein, 10 g cells were lysed in 100 ml lysis buffer (50 mM NaH₂PO₄, pH 8.0, 300 mM NaCl, 10 mM imidazole, 100 mg lysozyme (Serva, Germany) and 50 U Benzonase (Merck, Germany)) following by sonication 3 times with a ultrasonic processor (UP-200S, Dr. Hielscher GmbH, Germany) for 3 min at 5 kWsec⁻¹ in an ice/water bath. Cell debris was removed by centrifugation for 30 min at 4 °C and 25 000 x g.

Purification of recombinant MP-19

Recombinant expressed MP-19 was purified using Immobilized Metal Ion Affinity Chromatography (IMAC) and Reverse Phase Liquid Chromatography (RPLC). The chromatographic purification was realized using the AKTA-Explorer system (Pharmacia Biotech, Germany). For IMAC, a 1 ml Hi-Trap chelating column (Pharmacia Biotech) is used. Hi-Trap column was activated with 5 column volumes (cv) of 100 mM NiSO_4 , afterwards the column washed with 5 cv water to remove unbound Ni^{2+} . Column equilibration was performed with 5 cv of lysis buffer (50 mM NaH_2PO_4 , 300 mM NaCl, 10 mM imidazole, pH 8.0). Cell lysate results from 1 g *E. coli* cells was loaded onto column. Afterwards the column washed with lysis buffer to remove unbound protein. Protein were eluted using the following gradient program:

Step 1: 20 mM imidazole to 300 mM imidazole within 20 minutes, step 2: 300 mM imidazole to 500 mM imidazole within 10 minutes using buffer A (50 mM Tris, 300 mM NaCl, 20 mM imidazole, pH 6.0) and buffer B (50 mM Tris, 300 mM NaCl, 500 mM imidazole, pH 6.0). Flow rate of chromatography were 1 ml/min, detection were performed at 280 nm. Fractions were analyzed by immunological detection, shown in Figure 3. For further purification, positive fractions containing MP-19 were pooled and loaded onto a Resource RPC (3ml) column (Pharmacia Biotech). Column was equilibrated with buffer A (0.1 % trifluor acetic acid) and protein eluted with a linear gradient of buffer B (0.1 % TFA-90 % acetonitrile). Flow rate of chromatography were 3 ml/min, detection were performed at 215 nm.

Immunological Detection of MP-19

Immunological detection of recombinant MP-19 was performed by western blotting using a commercial available monoclonal mouse antibody against histidine-tag (Dianova, Germany, Cat. no. dia900) in combination with Western Light chemoluminescent detection system using the goat anti-mouse-AP antibody (Tropix, U.S.A.).

Activity assay for recombinant MP-19

A Serine/Threonine Phosphatase Assay System (Promega, Germany, Cat. no. V2460) was used to determine enzymatic activity of recombinant MP-19. This assay use a chemically synthesized phosphopeptide RRA(pT)VA which is a functional substrate for MP-19 phosphatase. The amount of free phosphate which is generated by MP-19 enzymatic reaction was measured by the absorbency of a molybdate:malachite green:phosphate complex (Ekman P and Jager O. (1993), Anal. Biochem. 214, 138-141, Deana A. D. et al. (1990), Biochimica et Biophysica Acta 1051, 199-202). Assays were performed as described by the manufacturer in PPase-2C buffer (50 mM imidazole, pH 7.2, 0.2 mM EGTA, 5 mM MgCl_2 , 0.02 % β -mercaptoethanol, 0.1 mg/ml BSA). To determine background of this assay, done pQE-16-dhfr (Diagen, Germany) was used, which is identical to pQE-MP-19 with exception that vector pQE-16 inserted a mouse dhfr gene instead of MP-19 phosphatase gene.

Results of activity assay of MP-19 shown in table 1. MP-19 has a significant activity in a MgCl_2 containing buffer, but no activity in a CaCl_2 containing buffer, which shows the requirement to Mg^{2+} . Inhibitors like okadaic acid (10 μM) shows no significant reduction of MP-19 activity. Control expression of the mouse dhfr gene shows no activity in the Serine/Threonine Phosphatase Assay System.

	A	B	C	D (Average A-C)
1	0.006	0.006	-0.006	0.000
2	0.024	0.029	0.023	0.025
3	0.304	0.291	0.298	0.298
4	0.612	0.594	0.597	0.601
5	1.080	1.140	1.137	1.119
6	0.021	0.006	0.018	0.015
7	0.025	0.043	0.018	0.029
8	0.038	0.020	0.018	0.025
9	0.030	0.012	0.013	0.018
10	0.151	0.108	0.174	0.144
11	0.146	0.147	0.139	0.144

Table 1: Activity test of MP-19

- A1-D1: Phosphate standard 0 pmol
 A2-D2: Phosphate standard 100 pmol
 A3-D3: Phosphate standard 500 pmol
 A4-D4: Phosphate standard 1000 pmol
 A5-D5: Phosphate standard 2000 pmol
 A6-D6: mouse dhfr gene with substrate (negative control)
 A7-D7: mouse dhfr gene without substrate (negative control)
 A8-D8: MP-19 with modified PPTase-2C buffer (5mM MgCl₂ is replaced by 5 mM CaCl₂) and substrate
 A9-D9: MP-19 with PPTase-2C buffer without substrate
 A10-D10: MP-19 with PPTase-2C buffer and substrate
 A11-D11: MP-19 with PPTase-2C buffer, substrate and 10 μ M okadaic acid

Annex to the description

Sequence listing

5

SEQ ID NO. 1

10

TACGGGCAGA	ACTGTCAAA	GGGCCCTCC	CACAGCAAAT	CTGGAGGTGG	50
GACAGGCGAG	GAACCAAGGT	CCCAGGCCCT	CAATGGGGAG	GCAGACCTG	100
AGGACTCAAC	TAGGGAACT	CCTTCACAA	AAAATGGCCC	CACAGCCAAG	150
GCCTACACAG	GCTTTTCCTC	CAACTCGGAA	CGTGGGACTG	AAGCAGGCCA	200
AGTTGGTGAG	CCTGGCATT	CCACTGGTGA	GGCTGGGCCT	TCCTGCTCTT	250
CAGCCTCTGA	CAAGCTGCC	CGAGTTGCTA	AGTCCAAGTT	CTTGAGGAC	300
AGTGAGGATG	AGTCAGATGA	GGCGGAGGAA	GAAGAGGAAG	ACAGTGAGGA	350
ATGCAGCGAG	GAAGAGGATG	GCTACAGCAG	TGAGGAGGCA	GAGAATGAGG	400
AAGATGAGGA	TGACACCGAG	GAGGCTGAAG	AGGACCATGA	AGAAGAAGAA	450
GAAGAGATGA	TGGTGCCAGG	GATGGAAGGC	AAAGAGGAGC	CTGGCTCTGA	500
CAGTGGTACA	ACAGCGGTGG	TGGCCCTGAT	ACGAGGGAAG	CAGTTGATTG	550
TAGCCAACGC	AGGAGACTCT	CGCTGTGTGG	TATCTGAGGC	TGGCAAAGCT	600
TTAGACATGT	CCTATGATCA	CAAAACGAGG	GATGAAGTAG	AACTAGCACG	650
CATCAAGAAT	GCTGGTGGCA	AGGTCACC			678

30

SEQ ID NO. 2

35

YQNCCHKGPP	HSKSGGGTGE	EPGSQGLNGE	AGPEDSTRET	PSQENGPTAK	50
AYTGFSNSE	RGTEAGQVGE	PGIPTGEAGP	SCSSASDKLP	RVAKSKFFED	100
SEDESDEAEE	EEEDSEECSE	EEDGYSSEE	ENEDEDDTE	EAEDEDEEE	150
EEMHVPMEG	KEEPPGSDSGT	TAVVALIRGK	QLIVANAGDS	RCVVSZAGKA	200
LDMSYDHPKE	DEVELARIKN	AGGKVT			226

40

45

50

55

SEQ ID NO. 3

ATGGGCTGCTTACTCTCTCCAGCCCAACACGGTGAAGTGGTCCGGGGACGGGGTGGGGGCC
 CGCCCTGCGGCTGCTCTAGGCTTCTCGCGGATGCAAGGCTGGCGGCTCCATGGAGGATG
 CTGACAACTCTATCTCTGAGTGGAGCAGTGAGACGCCATGCTTCTGTCTACGATGGACAT
 GGAGGGGAGGAAGTTGCCCTGTACTGTGCCAAATATCTTCTGTATATCATCAAAGATCAGAA
 GGCTTACAAGGAAGGCAAGCTACAGAAGGCTTTAGAAGATGCGCTTCTGGCTATTGACGCCA
 AATTGACCACTGAAGAACTGATTAAAGAGCTGGGCACAGATTGCAGGGCGACCCACTGAGGAT
 GAAGATGAAAAAGAAAAAGTAGCTGATGAAGATGATGTGGACAATGAGGAGGCTGCACTGCT
 GCATGAAGAGGCTACCATGACTATTGAAGAGCTGCTGACACGCTACGGGCGAAGCTGTCA
 AGGGCCCTTCCACACGCAAACTCTGGAGGTGGGACAGGGGAGGAACAGGGTCCCAGGGCCCTC
 AATGGGGAGGGCAGGACCTGAGGACTCAACTAGGGAACTCCTTCAAGAAAAATGGCCCCAC
 AGCCAAAGGCTTACACAGGCTTTTCTCCAACCTCGGAACGTGGGACTGAGGCGAGGCCAAGTTG
 CTGAGCTTGGGATTCCCACTGGTGGGCTGGGCCCTTCTGCTCTTCAAGCTCTTGACAAGCTG
 CTTCAGATTTGCTAAGTCCAACTTTCTTGAAGACAGTGAGGATGAGTCAGATGAGGCGGAGGA
 AGAAGAGGAAGACAGTGAAGAAATGCAGCGAGGAAGAGGATGGCTACAGCAGTGAGGAGGCGAG
 AGAATGAGGAAGATGAGGATGACACCGAGGAGGCTGAAGAGGACGATGAAGAAGAAGAAGAA
 GAGATGATGCTGCCAGGGATGGAAGGCAAGAGGAGCCTGGCTCTGACAGTGGTACAACAGC
 GGTGGTGGCCCTGATACGAGGGGAAGCAGTTGATTGTAGCCAACCGAGGAGACTCTCGCTGTG
 TGGTATCTGAGGCTGGCAAGGCTTTAGACATGTCTTATGATCACAACACGAGGATGAAGTA
 GAACTAGGCACGCTCAAGAAATGCTGGTGGCAAGGTCACCATGGATGGCGGAGTCAACGGGGG
 CCTCAACCTCTCCAGAGCCATTGGGGACCACTTCTATAAGAGAAACAGAACCTGCCACCTG
 AGGAACAGATGATTTCAGCCCTTCTGACATCAAGGTGCTGACTCTCACTGACGACCATGAA
 TTCATGGTCATTGCTGTGATGGCATCTGGAATGATGAGGAGCGAGGAAGTTGTAGATTT
 CATTCAATCAAAGATCAGCAGCCTGATGAAAATGGGGAGCTTCGGTTATTGTCTATCCATTG
 TGGAAAGAGCTCTGGATCAGTGCTGGCACCAGACACTCTTGGGGATGGTACAGGGTGTGAC
 AACATGAGCTGCATCATCTTTGTTCAAGCCCCGAAACACAGCAGAGCTCCAGCGACAGAG
 TGGCAAGCGAAACTAGAGGAGGTGCTCTCTACTGAGGGGGCTGAAGAAAAATGGCAACAGCG
 ACAAGAAGAAGAAGGCCAAGCGAGACTAG

SEQ ID NO. 4

MGAYLSQPNP VKCSGGGVGA PRPLPYGFS AMGGWRVSM DARNCEPILD SETAMFSYVD
 GHGGEEVALY CAXYLPDIK DOKAYKEGL OKALEDAFLA IDAKLTTEEV IKELACIAGR
 PTEDEDENEK VACEDDVONE EAALLHEEAT MIEELLTRY GONCKRGPPH SKSGGGTSEE
 PGSGGLNGEA GPEDSTRETP SQENGFATAK YGFSMSNER GTEAGGVGEP GTPTSAGAPS
 CSASDMLER VAKSFFEDS EUESSEAEF EDSGSESE EHGSSSEAE NEDEEDITE
 AEDDEEEF EMWPGMECK EEPGSDGFT AVVALIRGQ LTVANAGDSR CVSEAGVAL
 DMSYDHKEPD EVELARIKNA GSKVNMDSRV NGGLNSRAI GSHFYKNNN LPPEEQISA
 LPMIKVLTIT DDHEPMVIAC GDIWNVMSQ EVVDFIQSI SROENGLR LLSSIVEELL
 DQCLAPDTSG DGTGCDNMTI IIECFKPNRT AELQFESGR KLEEVLTSEG AEENGNSRKK
 KKAARD

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Biopharm GmbH
 (B) STREET: Czernyring 22
 (C) CITY: Heidelberg
 (E) COUNTRY: Germany
 (F) POSTAL CODE (ZIP): 69115

(ii) TITLE OF INVENTION: Nucleic acid encoding a novel human protein phosphatase

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: EP 98107346.3

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 678 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TACGGGCAGA ACTGTCACAA GGGCCCTCCC CACAGCAAAT CTGGAGGTGG GACAGGCGAG	60
GAACCAGGGT CCCAGGCGCT CAATGGGGAG GCAGGACCTG AGGACTCAAC TAGGGAAACT	120

CCTTCACAAG AAAATGGCCC CACAGCCAAG GCCTACACAG GCTTTTCCTC CAACTCGGAA 180
 CGTGGGACTG AAGCAGGCCA AGTTGGTGAG CTTGGCATTC CCCTGGTGA GGCTGGGCCT 240
 TCCTGCTCTT CAGCCTCTGA CAAGCTGCTT CGAGTTGCTA AGTCCAAGTT CTTTGAGGAC 300
 AGTGAGGATG AGTCAGATGA GCGGAGGAA GAAGAGGAAG ACAGTGAGGA ATGCAGCGAG 360
 GAAGAGGATG GCTACAGCAG TGAGGAGGCA GAGAATGAGG AAGATGAGGA TGACACCGAG 420
 GAGGCTGAAG AGGACGATGA AGAAGAAGAA GAAGAGATGA TGSTGCCAGG GATGGAAGGC 480
 AAAGAGGAGC CTGGCTCTGA CAGTGGTACA ACAGCGGTGG TGGCCCTGAT ACGAGGGAAG 540
 CAGTTGATTG TAGCCAACGC AGGAGACTCT CGCTGTGTGG TATCTGAGGC TGGCAAAGCT 600
 TTAGACATGT CCTATGATCA CAAACCAGAG GATGAAGTAG AACTAGCAGC CATCAAGAAT 660
 GCTGGTGGCA AGGTCACC 678

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 226 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Tyr Gly Gln Asn Cys His Lys Gly Pro Pro His Ser Lys Ser Gly Gly
 1 5 10
 Gly Thr Gly Glu Glu Pro Gly Ser Gln Gly Leu Asn Gly Glu Ala Gly
 20 25 30
 Pro Glu Asp Ser Thr Arg Glu Thr Pro Ser Gln Glu Asn Gly Pro Thr
 35 40 45
 Ala Lys Ala Tyr Thr Gly Phe Ser Ser Asn Ser Glu Arg Gly Thr Glu

	50	55	60
5	Ala Gly Gln Val Gly Glu Pro Gly Ile Pro Thr Gly Glu Ala Gly Pro 65 70		
	Ser Cys Ser Ser Ala Ser Asp Lys Leu Pro Arg Val Ala Lys Ser Lys 85 90		
10	Phe Phe Glu Asp Ser Glu Asp Glu Ser Asp Glu Ala Glu Glu Glu 100 105		
	Glu Asp Ser Glu Glu Cys Ser Glu Glu Glu Asp Gly Tyr Ser Ser Glu 115 120 125		
15	Glu Ala Glu Asn Glu Glu Asp Glu Asp Asp Thr Glu Glu Ala Glu Glu 130 135 140		
	Asp Asp Glu Glu Glu Glu Glu Met Met Val Pro Gly Met Glu Gly 145 150 155 160		
20	Lys Glu Glu Pro Gly Ser Asp Ser Gly Thr Thr Ala Val Val Ala Leu 165 170 175		
	Ile Arg Gly Lys Gln Leu Ile Val Ala Asn Ala Gly Asp Ser Arg Cys 180 185 190		
25	Val Val Ser Glu Ala Gly Lys Ala Leu Asp Met Ser Tyr Asp His Lys 195 200 205		
	Pro Glu Asp Glu Val Glu Leu Ala Arg Ile Lys Asn Ala Gly Gly Lys 210 215 220		
30	Val Thr 225		

(2) INFORMATION FOR SEQ ID NO: 3:

- 35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1641 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- 40 (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- 45 (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (F) TISSUE TYPE: human placenta
- 50
- 55

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(X1) SEQUENCE DESCRIPTION, SEQ ID NO: 3:

	ATGGGTGCCT ACCTCTCCCA GCCAACACG GTGAAGTGCT CCGGGACGG GGTGGCGGCC	60
5	CCGCGCCTGC CGCTGCCCTA CGGCTTCTCC GCCATGCAAG GCTGGCGCGT CTCCATGGAG	120
	GATGCTCACA ACTGTATTCC TGAGCTGGAC AGTGAGACAG CCATGTTTTT TGTCTACGAT	180
	GGACATGGAG GGGAGGAAGT TGCCTTGATC TGTGCCAAAT ATCTTCCTGA TATCATCAAA	240
10	GATCAGAAGG CCTACAAGGA AGGCAAGCTA CAGAAGGCTT TAGAAGATGC CTCTTTGGCT	300
	ATTGACGCCA AATTGACCAC TGAAGAAGTC ATTAAGAGC TGCCACAGAT TGCAGGGCGA	360
	CCCCTGAGG ATGAAGATGA AAAAGAAAAA GTAGCTGATG AAGATGATGT GGACAATGAG	420
15	GAGGCTGCAC TGCTGCATGA AGAGGCTACC ATGACTATTG AAGAGCTGCT GACACGTAC	480
	GGGCAGAACT GTCAACAAGG CCTCCCCAC AGCAAACTCTG GAGGTGGGAC AGGCGAGGAA	540
	CCAGGGTCCC AGGGCCTCAA TGGGGAGGCA GGACCTGAGG ACTCAACTAG GGAACTCCT	600
20	TCACAAGAAA ATGGCCCCAC AGCCAAGGCC TACACAGGCT TTTCTCCAA CTCGGAACGT	660
	GGGACTGAGG CAGGCCAAGT TGGTGAGCCT GGCATTCCCA CTGGTGAGGC TGGGCCTTCC	720
	TGCTCTTCAG CCTCTGACAA GCTGCCTCGA GTTGCTAAGT CCAAGTTCTT TGAGGACAGT	780
25	GAGGATGAGT CAGATGAGGC GGAGGAAGAA GAGGAAGACA GTGAGGAATG CAGCGAGGAA	840
	GAGGATGGCT ACAGCAGTGA GGAGGCAGAG AATGAGGAAG ATGAGGATGA CACCGAGGAG	900
	GCTGAAGAGG ACGATGAAGA AGAAGAAGAA GAGATGATGG TGCCAGGGAT GGAAGGCAAA	960
30	GAGGAGCCTG GCTCTGACAG TGGTACAACA GCGGTGGTGG CCCTGATACG AGGGAAGCAG	1020
	TTGATTGTAG CCAACGCAGG AGACTCTCGC TGTGTGGTAT CTGAGGCTGG CAAAGCTTTA	1080
	GACATGTCCT ATGATCACAA ACCAGAGGAT GAAGTAGAAC TAGCACGCAT CAAGAATGCT	1140
35	GGTGGCAAGG TCACCATGGA TGGGCGAGTC AACGGGGGCC TCAACCTCTC CAGAGCCATT	1200
	GGGGACCACT TCTATAAGAG AAACAAGAAC CTGCCACCTG AGGAACAGAT GATTTCAGCC	1260
	CTTCCTGACA TCAAGGTGCT GACTCTCACT GACGACCATG AATTCATGGT CATTGCGTGT	1320
40	GATGGCATCT GGAATGTGAT GAGCAGCCAG GAAGTTGTAG ATTTTCATTCA ATCAAGATAC	1380

AGCCAGCGTG ATGAAAATGG GGAGCTTCGG TTATTGTCAT CCATTGTGGA AGAGCTGCTG 1440
 GATCAGTGCC TGGCACCAGA CACTTCTGGG GATGGTACAG GGTGTGACAA CATGACCTGC 1500
 ATCATCATTT GCTTCAAGCC CCGAAACACA GCAGAGCTCC AGCCAGAGAG TGGCAAGOGA 1560
 AAACGTAGAG AGGTGCTCTC TACTGAGGGG GCTGAAGAAA ATGGCAACAG CGACAAGAAG 1620
 AAGAAGGCCA AGCGAGACTA G 1641

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 546 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Gly Ala Tyr Leu Ser Gln Pro Asn Thr Val Lys Cys Ser Gly Asp
 1 5 10 15
 Gly Val Gly Ala Pro Arg Leu Pro Leu Pro Tyr Gly Phe Ser Ala Met
 20 25 30
 Gln Gly Trp Arg Val Ser Met Glu Asp Ala His Asn Cys Ile Pro Glu
 35 40 45
 Leu Asp Ser Glu Thr Ala Met Phe Ser Val Tyr Asp Gly His Gly Gly
 50 55 60
 Glu Glu Val Ala Leu Tyr Cys Ala Lys Tyr Leu Pro Asp Ile Ile Lys
 65 70 75 80
 Asp Gln Lys Ala Tyr Lys Glu Gly Lys Leu Gln Lys Ala Leu Glu Asp
 85 90 95
 Ala Phe Leu Ala Ile Asp Ala Lys Leu Thr Thr Glu Glu Val Ile Lys
 100 105 110

Glu Leu Ala Gln Ile Ala Gly Arg Pro Thr Glu Asp Glu Asp Glu Lys
 115 120 125
 5 Glu Lys Val Ala Asp Glu Asp Val Asp Asn Glu Glu Ala Ala Leu
 130 135 140
 Leu His Glu Glu Ala Thr Met Thr Ile Glu Glu Leu Leu Thr Arg Tyr
 145 150 155 160
 10 Gly Gln Asn Cys His Lys Gly Pro Pro His Ser Lys Ser Gly Gly Gly
 165 170 175
 Thr Gly Glu Glu Pro Gly Ser Gln Gly Leu Asn Gly Glu Ala Gly Pro
 180 185 190
 15 Glu Asp Ser Thr Arg Glu Thr Pro Ser Gln Glu Asn Gly Pro Thr Ala
 195 200 205
 20 Lys Ala Tyr Thr Gly Phe Ser Ser Asn Ser Glu Arg Gly Thr Glu Ala
 210 215 220
 Gly Gln Val Gly Glu Pro Gly Ile Pro Thr Gly Glu Ala Gly Pro Ser
 225 230 235 240
 25 Cys Ser Ser Ala Ser Asp Lys Leu Pro Arg Val Ala Lys Ser Lys Phe
 245 250 255
 Phe Glu Asp Ser Glu Asp Glu Ser Asp Glu Ala Glu Glu Glu Glu
 260 265 270
 30 Asp Ser Glu Glu Cys Ser Glu Glu Glu Asp Gly Tyr Ser Ser Glu Glu
 275 280 285
 Ala Glu Asn Glu Glu Asp Glu Asp Asp Thr Glu Glu Ala Glu Glu Asp
 290 295 300
 35 Asp Glu Glu Glu Glu Glu Glu Met Met Val Pro Gly Met Glu Gly Lys
 305 310 315 320
 40 Glu Glu Pro Gly Ser Asp Ser Gly Thr Thr Ala Val Val Ala Leu Ile
 325 330 335
 Arg Gly Lys Gln Leu Ile Val Ala Asn Ala Gly Asp Ser Arg Cys Val
 340 345 350
 45 Val Ser Glu Ala Gly Lys Ala Leu Asp Met Ser Tyr Asp His Lys Pro
 355 360 365
 Glu Asp Glu Val Glu Leu Ala Arg Ile Lys Asn Ala Gly Gly Lys Val
 370 375 380
 50
 55

Thr Met Asp Gly Arg Val Asn Gly Gly Leu Asn Leu Ser Arg Ala Ile
 385 390 395 400
 5 Gly Asp His Phe Tyr Lys Arg Asn Lys Asn Leu Pro Pro Glu Glu Gln
 405 410 415
 Met Ile Ser Ala Leu Pro Asp Ile Lys Val Leu Thr Leu Thr Asp Asp
 420 425 430
 10 His Glu Phe Met Val Ile Ala Cys Asp Gly Ile Trp Asn Val Met Ser
 435 440 445
 Ser Gln Glu Val Val Asp Phe Ile Gln Ser Lys Ile Ser Gln Arg Asp
 450 455 460
 15 Glu Asn Gly Glu Leu Arg Leu Leu Ser Ser Ile Val Glu Glu Leu Leu
 465 470 475 480
 20 Asp Gln Cys Leu Ala Pro Asp Thr Ser Gly Asp Gly Thr Gly Cys Asp
 485 490 495
 Asn Met Thr Cys Ile Ile Ile Cys Phe Lys Pro Arg Asn Thr Ala Glu
 500 505 510
 25 Leu Gln Pro Glu Ser Gly Lys Arg Lys Leu Glu Glu Val Leu Ser Thr
 515 520 525
 Glu Gly Ala Glu Glu Asn Gly Asn Ser Asp Lys Lys Lys Lys Ala Lys
 530 535 540
 30 Arg Asp
 545

35

Claims

- 40 1. A nucleic acid comprising a nucleotide sequence encoding a human serine/threonine phosphatase or a functional fragment thereof that is capable of dephosphorylating serine or threonine residues, wherein the nucleotide sequence comprises:
- 45 (a) the nucleotides as shown in SEQ ID NO. 1 or SEQ ID NO. 3, or
 (b) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequence of (a); or
 (c) an allelic derivative of the sequences of (a) or (b); or
- 50 (d) a DNA sequence which is capable of hybridizing to the sequences in (a), (b) and (c), and encoding a protein containing the amino acid sequence as depicted in SEQ ID NO. 2 or SEQ ID NO. 4; or
- 55 (e) a nucleotide sequence which is capable of hybridizing to the DNA sequences in (a), (b), (c) and (d), and encoding a protein having essentially the same biological properties as the protein defined in (d).
2. The nucleic acid according to claim 1, wherein the nucleotide sequence is a vertebrate DNA sequence, a mammalian sequence, preferably a primate, human, porcine, or rodent, preferably a rat or rabbit, DNA sequence.

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3. A recombinant molecule comprising a nucleic acid according to claim 1 or 2
4. The recombinant molecule according to claim 3, wherein said nucleic acid sequence is functionally linked to an expression-control sequence
5. A host containing the nucleic acid according to claim 1 or 2, or the recombinant molecule according to claim 3 or 4
6. The host according to claim 5, which is a bacterium, a fungus, a plant cell, an animal or a human cell
7. A process for the production of a PP2C-like protein comprising cultivating a host according to claim 5 or 6 and recovering said PP2C-like protein from the culture
8. A PP2C-like protein or a biologically active fragment thereof encoded by a nucleic acid according to claim 1 or 2 or by a recombinant molecule according to claim 3 or 4
9. The protein according to claim 8, comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4
10. An agonist as a substitute for the protein of claim 8 or 9
11. An antagonist directed to the protein of claim 8 or 9
12. A pharmaceutical composition containing the protein or a biologically active fragment thereof according to claim 8 and 9 or the agonist according to claim 10 or the antagonist according to claim 11, and optionally, a pharmaceutically acceptable carrier and/or diluent
13. The pharmaceutical composition according to claim 12 for the treatment of leukemia, brain cancer, breast cancer, prostate cancer, Alzheimer's disease, Huntington's disease, Parkinson's disease, and epilepsy, and of disorders of the reproductive system, or for the regulation of spermatogenesis or the maturation of mammalian germ cells
14. An antibody or antibody fragment which is capable of specifically binding to the protein of claim 8 and 9 or to the agonist of claim 10 or to the antagonist of claim 11
15. The antibody according to claim 14, which is a monoclonal antibody
16. Use of an antibody or antibody fragment according to claim 14 or 15 for detecting the protein or a biologically active fragment thereof as defined in claim 8 or 9
17. A diagnostic kit containing the agonist according to claim 10 or the antagonist according to claim 11 or the antibody or antibody fragment according to claim 14 or 15

Fig. 1

MP19-PCR	YQCNCHKGPP	HSKSGGGTGE	EPGQQGLNGE	AGPEDSTRET	PSQENGPTAK	50
PP2C-Human	MGAFLDKPKM	EKNNAQGGG-	---NGLRYG	LSSMQGMRVE	MEDAHAVIG	45
PP2C-Rabbit	MGAFLDKPKM	EKNNAQGGG-	---NGLRYG	LSSMQGMRVE	MEDAHAVIG	45
PP2C-Rat	MGAFLDKPKM	EKNNAQGGG-	---NGLRYG	LSSMQGMRVE	MEDAHAVIG	45
	*	*	*	*	*	
MP19-PCR	AYTGPFSSNSE	RCTEAGQVGE	PGIPTGEAGP	SCSSASDKLP	RVAKSKFFED	100
PP2C-Human	LPSSGLESMWF	FAVYDGHAG-	----SQVAK	YCC--EHLLO	HITNNQDFRG	87
PP2C-Rabbit	LPSSGLESMWF	FAVYDGHAG-	----SQVAK	YCC--EHLLO	HITNNQDFRG	87
PP2C-Rat	LPSSGLESMWF	FAVYDGHAG-	----SQVAK	YCC--EHLLO	HITNNQDFRG	87
	*	*	*	*	*	
MP19-PCR	SEDESDEAEE	EEEDSEECSE	EEDGYSSEEA	ENEEDDEDTE	EAEDDEEEEE	150
PP2C-Human	SAGAP-SVEN	VKNIGI-----	-RTGF-----	-----LEID		109
PP2C-Rabbit	SAGAP-SVEN	VKNIGI-----	-RTGF-----	-----LEID		109
PP2C-Rat	SAGAP-SVEN	VKNIGI-----	-RTGF-----	-----LEID		109
	*	*	*	*	*	
MP19-PCR	EEHMYVPGNEG	KEEPGSDSOT	TAVVALIRGK	QLIVANAGDS	RCVVSEAGKA	200
PP2C-Human	EHMRV--MSE	KKHGADRSGS	TAVGVLSIPQ	HTYFINGGDS	RGLLCRNKKV	157
PP2C-Rabbit	EHMRV--MSE	KKHGADRSGS	TAVGVLSIPQ	HTYFINGGDS	RGLLCRNKKV	157
PP2C-Rat	EHMRV--MSE	KKHGADRSGS	TAVGVLSIPQ	HTYFINGGDS	RGLLCRNKKV	157
	*	*	*	*	*	
	*	*	*	*	*	
	*	*	*	*	*	
	*	*	*	*	*	
MP19-PCR	LDMSYDHKPE	DEVILARIKN	AGGKVT			226
PP2C-Human	HFTTQDHKPS	NPLEKERIQN	AGGSVM			183
PP2C-Rabbit	HFTTQDHKPS	NPLEKERIQN	AGGSVM			183
PP2C-Rat	HFTTQDHKPS	NPLEKERIQN	AGGSVM			183
	****	* * *	***			

Figure 2

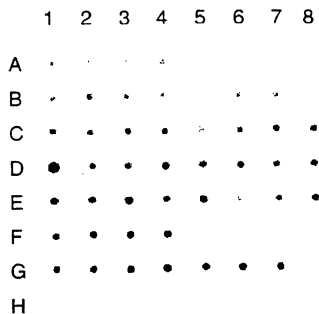
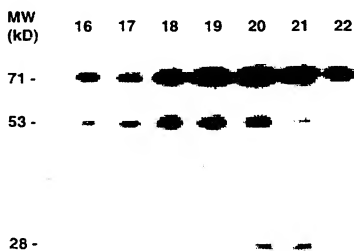


Figure 3



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G01N 33/577

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Remarks:

The applicant has subsequently filed a sequence listing and declared, that it includes no new mat

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(54) Nucleic acid encoding a human protein phosphatase

(57) The present invention relates to nucleic acids encoding a novel human protein phosphatase of the family of protein serine/threonine phosphatases. In particular, it relates to novel DNA sequences encoding serine/threonine protein phosphatase, to expression plasmids containing said nucleic acids, to host organisms transformed by said expression plasmids, to the production of said protein by culturing said transformant, to antibodies specifically binding to said phosphatase and to agonists and/or antagonists for said protein, and to antisense MP-19 nucleic acid. Furthermore, the invention relates to serine or threonine residues and epitopes comprising said residues dephosphorylated by said protein and pharmaceutical compositions comprising said protein or agonists or antagonists thereof for the treatment of diseases influenced by changes in phosphorylation which controls e.g. cell proliferation and/or differentiation, to diagnostic kits and to *in vitro* diagnostic methods for the detection of phosphorylation dependent diseases such as e.g. cancer.

Fig. 1

[illegible]

HP1-PCR	HP100-SAGE	HP100-Seq	HP100-Seq	HP100-Seq	HP100-Seq	HP100-Seq
HP100-Seq	SAGE-Seq	HP100-Seq	HP100-Seq	HP100-Seq	HP100-Seq	HP100-Seq
HP100-Seq	SAGE-Seq	HP100-Seq	HP100-Seq	HP100-Seq	HP100-Seq	HP100-Seq
HP100-Seq	SAGE-Seq	HP100-Seq	HP100-Seq	HP100-Seq	HP100-Seq	HP100-Seq

SP-1-103	SP-1-104	SP-1-105	SP-1-106	SP-1-107	SP-1-108	SP-1-109	SP-1-110	SP-1-111	SP-1-112	SP-1-113	SP-1-114	SP-1-115	SP-1-116	SP-1-117	SP-1-118	SP-1-119	SP-1-120	SP-1-121	SP-1-122	SP-1-123	SP-1-124	SP-1-125	SP-1-126	SP-1-127	SP-1-128	SP-1-129	SP-1-130	SP-1-131	SP-1-132	SP-1-133	SP-1-134	SP-1-135	SP-1-136	SP-1-137	SP-1-138	SP-1-139	SP-1-140	SP-1-141	SP-1-142	SP-1-143	SP-1-144	SP-1-145	SP-1-146	SP-1-147	SP-1-148	SP-1-149	SP-1-150	SP-1-151	SP-1-152	SP-1-153	SP-1-154	SP-1-155	SP-1-156	SP-1-157	SP-1-158	SP-1-159	SP-1-160	SP-1-161	SP-1-162	SP-1-163	SP-1-164	SP-1-165	SP-1-166	SP-1-167	SP-1-168	SP-1-169	SP-1-170	SP-1-171	SP-1-172	SP-1-173	SP-1-174	SP-1-175	SP-1-176	SP-1-177	SP-1-178	SP-1-179	SP-1-180	SP-1-181	SP-1-182	SP-1-183	SP-1-184	SP-1-185	SP-1-186	SP-1-187	SP-1-188	SP-1-189	SP-1-190	SP-1-191	SP-1-192	SP-1-193	SP-1-194	SP-1-195	SP-1-196	SP-1-197	SP-1-198	SP-1-199	SP-1-200	SP-1-201	SP-1-202	SP-1-203	SP-1-204	SP-1-205	SP-1-206	SP-1-207	SP-1-208	SP-1-209	SP-1-210	SP-1-211	SP-1-212	SP-1-213	SP-1-214	SP-1-215	SP-1-216	SP-1-217	SP-1-218	SP-1-219	SP-1-220	SP-1-221	SP-1-222	SP-1-223	SP-1-224	SP-1-225	SP-1-226	SP-1-227	SP-1-228	SP-1-229	SP-1-230	SP-1-231	SP-1-232	SP-1-233	SP-1-234	SP-1-235	SP-1-236	SP-1-237	SP-1-238	SP-1-239	SP-1-240	SP-1-241	SP-1-242	SP-1-243	SP-1-244	SP-1-245	SP-1-246	SP-1-247	SP-1-248	SP-1-249	SP-1-250	SP-1-251	SP-1-252	SP-1-253	SP-1-254	SP-1-255	SP-1-256	SP-1-257	SP-1-258	SP-1-259	SP-1-260	SP-1-261	SP-1-262	SP-1-263	SP-1-264	SP-1-265	SP-1-266	SP-1-267	SP-1-268	SP-1-269	SP-1-270	SP-1-271	SP-1-272	SP-1-273	SP-1-274	SP-1-275	SP-1-276	SP-1-277	SP-1-278	SP-1-279	SP-1-280	SP-1-281	SP-1-282	SP-1-283	SP-1-284	SP-1-285	SP-1-286	SP-1-287	SP-1-288	SP-1-289	SP-1-290	SP-1-291	SP-1-292	SP-1-293	SP-1-294	SP-1-295	SP-1-296	SP-1-297	SP-1-298	SP-1-299	SP-1-300	SP-1-301	SP-1-302	SP-1-303	SP-1-304	SP-1-305	SP-1-306	SP-1-307	SP-1-308	SP-1-309	SP-1-310	SP-1-311	SP-1-312	SP-1-313	SP-1-314	SP-1-315	SP-1-316	SP-1-317	SP-1-318	SP-1-319	SP-1-320	SP-1-321	SP-1-322	SP-1-323	SP-1-324	SP-1-325	SP-1-326	SP-1-327	SP-1-328	SP-1-329	SP-1-330	SP-1-331	SP-1-332	SP-1-333	SP-1-334	SP-1-335	SP-1-336	SP-1-337	SP-1-338	SP-1-339	SP-1-340	SP-1-341	SP-1-342	SP-1-343	SP-1-344	SP-1-345	SP-1-346	SP-1-347	SP-1-348	SP-1-349	SP-1-350	SP-1-351	SP-1-352	SP-1-353	SP-1-354	SP-1-355	SP-1-356	SP-1-357	SP-1-358	SP-1-359	SP-1-360	SP-1-361	SP-1-362	SP-1-363	SP-1-364	SP-1-365	SP-1-366	SP-1-367	SP-1-368	SP-1-369	SP-1-370	SP-1-371	SP-1-372	SP-1-373	SP-1-374	SP-1-375	SP-1-376	SP-1-377	SP-1-378	SP-1-379	SP-1-380	SP-1-381	SP-1-382	SP-1-383	SP-1-384	SP-1-385	SP-1-386	SP-1-387	SP-1-388	SP-1-389	SP-1-390	SP-1-391	SP-1-392	SP-1-393	SP-1-394	SP-1-395	SP-1-396	SP-1-397	SP-1-398	SP-1-399	SP-1-400	SP-1-401	SP-1-402	SP-1-403	SP-1-404	SP-1-405	SP-1-406	SP-1-407	SP-1-408	SP-1-409	SP-1-410	SP-1-411	SP-1-412	SP-1-413	SP-1-414	SP-1-415	SP-1-416	
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Protein	Accession	Length	Weight	PI
Protein	Protein	Protein	Protein	Protein
Protein	Protein	Protein	Protein	Protein
Protein	Protein	Protein	Protein	Protein
Protein	Protein	Protein	Protein	Protein

Description

The present invention relates to nucleic acids encoding a novel human protein phosphatase of the protein serine/threonine phosphatase family. In particular, it relates to novel DNA sequences encoding a serine/threonine protein phosphatase, to expression plasmids containing said nucleic acids, to host organisms transformed by said expression plasmids, to the production of said protein by culturing said transformant, to antibodies specifically binding to said phosphatase and to agonists and/or antagonists for said protein. Furthermore, the invention relates to serine or threonine residues and epitopes comprising said residues dephosphorylated by said protein and pharmaceutical compositions comprising said protein or agonists or antagonists thereof for the treatment of diseases influenced by changes in phosphorylation which controls e.g. cell proliferation and/or differentiation, to diagnostic kits and to *in vitro* diagnostic methods for the detection of phosphorylation dependent diseases such as e.g. cancer.

Protein phosphorylation-dephosphorylation is a universal mechanism by which different cellular events are regulated. The serine/threonine-specific phosphatases have been classified into four main types according to their *in vitro* specificity for selected substrates and sensitivity to activators and inhibitors (Ingebritsen, T.S. and Cohen, P. (1983) *Eur. J. Biochem.* 182, 255-261). Sequence analysis revealed that they can be classified into two major gene families. The first one includes type 1 (PP1), type 2A (PP2A), and type 2B (PP2B) phosphatases, which share 37 to 59 % sequence identity (Barton, G.J. et al., (1994) *Eur. J. Biochem.* 220, 225-237) in their catalytic domains and are inhibited by okadaic acid (Bialojan, C., and Takai, A. (1988) *Biochem. J.* 256, 283-290). The second family, the Mg^{2+} -dependent phosphatases, also designated type 2C (PP2C), share little sequence similarity with the first family and are insensitive to okadaic acid. cDNA sequences of PP2C α and β from mammalian sources showed > 90 % identity. PP2Cs have been implicated in the regulation of fatty acid and cholesterol biosynthesis (Moore, F. et al. (1991) *Eur. J. Biochem.* 199, 691-697) and heat shock response (Maeda et al. (1993) *Mol. Cell. Biol.* 13, 5408-5417, Shiozaki, K. et al. (1994) *Mol. Cell. Biol.* 14, 3742-375).

The technical problem underlying the present invention is to provide a new human PP2C-like protein phosphatase which is distinct from the other PP2Cs.

The solution to the above technical problem is achieved by providing the embodiments characterized in the claims. Other features and advantages of the invention will be apparent from the description of the preferred embodiments and drawings.

The sequence listings and drawings will now be briefly described.

SEQ ID NO. 1 shows the nucleotide sequence of MP-19, a DNA sequence derived from human placenta.

SEQ ID NO. 2 shows the amino acid sequence of MP-19 as deduced from SEQ ID NO. 1.

SEQ ID NO. 3 shows the nucleotide sequence of MP-19 full-length cDNA derived from human placenta.

SEQ ID NO. 4 shows the amino acid sequence of MP-19 as deduced from SEQ ID NO. 3.

The figures show:

Figure 1 shows the alignment of the amino acid sequence of MP-19 with some related proteins of the PP2C family. The asterisk (*) indicates the position of identical amino acids of the compared amino acid sequences.

MP19-PCR: amino acid sequence of MP-19 (SEQ ID NO. 2)
PP2C-Human: human protein phosphatase 2C alpha (Accession No. S87759)
PP2C-Rabbit: rabbit protein phosphatase 2C alpha (Accession No. S87757)
PP2C-Rat: rat protein phosphatase 2C (Accession No. J04503)

Figure 2 is a human RNA master blot. The RNA-dot blot analysis shows hybridization of MP-19 PCR probe with different human RNA samples.

A1 whole brain, A2 amygdala, A3 caudate nucleus, A4 cerebellum, A5 cerebral cortex, A6 frontal lobe, A7 hippocampus, A8 medulla oblongata, B1 occipital lobe, B2 putamen, B3 substantia nigra, B4 temporal lobe, B5 thalamus, B6 subthalamic nucleus, B7 spinal cord, B8 blank, C1 heart, C2 aorta, C3 skeletal muscle, C4 colon, C5 bladder, C6 uterus, C7 prostate, C8 stomach, D1 testis, D2 ovary, D3 pancreas, D4 pituitary gland, D5 adrenal gland, D6 thyroid gland, D7 salivary gland, D8 mammary gland, E1 kidney, E2 liver, E3 small intestine, E4 spleen, E5 thymus, E6 peripheral leukocyte, E7 lymph node, E8 bone marrow, F1 appendix, F2 lung, F3 trachea, F4 placenta, F5-F8 blank, G1 fetal brain, G2 fetal heart, G3 fetal kidney, G4 fetal liver, G5 fetal spleen, G6 fetal thymus, G7 fetal lung, G8 blank, H1 yeast total RNA, H2 yeast tRNA, H3 *E. coli* rRNA, H4 *E. coli* DNA, H5 poly r(A), H6-H8 human DNA.

Figure 3 shows the detection of MP-19 after IMAC. The Western analysis was performed after purification of MP-19 using Immobilized Metal Ion Affinity Chromatography (IMAC). Positive signals were obtained from fraction 16 to fraction 22.

5 The amino acid sequence alignment of MP-19 with sequences of different PP2Cs (shown in Figure 1) demonstrates the homology of MP-19 to the PP2C family but implicate also that MP-19 belongs to a new protein phosphatase group. The homology of the derived MP-19 amino acid sequence (aa 1 - aa 226) to PP2C from human, rabbit and rat displays a sequence homology of 21.2 %. Moreover a partial sequence of MP-19 (amino acid sequence 158 - 226) which is in the shown alignment not disrupted by gaps indicates a sequence homology of 39.1 % to the compared
10 PP2Cs.

The present invention relates particularly to a novel serine/threonine protein phosphatase and, preferably, provides DNA sequences contained in the corresponding gene.

Such sequences include nucleotide sequences as illustrated in SEQ ID NO. 1 and SEQ ID NO. 3, allelic derivatives of said sequences and DNA sequences degenerated as a result of the genetic code for said sequences. It also includes
15 DNA sequences hybridizing under stringent conditions with the DNA sequence mentioned above. It further includes antisense nucleic acid, preferably antisense MP-19 nucleic acid, directed to the above defined nucleic acid. The terms "nucleic acid sequence" and "nucleotide sequence" refers to DNA or RNA or heterooligomeric sequences, which may be double- or single-stranded.

Although said allelic, degenerate and hybridizing sequences may have structural divergences due to naturally occurring mutations, such as small deletions or substitutions, they will usually still exhibit essentially the same useful properties, allowing their use in basically the same medical or diagnostic applications.

According to the present invention, the term "hybridization" means conventional hybridization conditions, preferably conditions with a salt concentration of 6 x SSC at 62°C to 66°C followed by a one-hour wash with 0.6 x SSC, 0.1 % SDS at 62°C to 66°C.

25 Important biological embodiments of the present invention are DNA sequences of the above and obtainable from vertebrates, preferably mammals such as pig and from rodents such as rat, and in particular from primates such as humans.

Particularly preferred embodiments of the present invention are the DNA sequence termed MP-19 which are shown in SEQ ID NO. 1 and SEQ ID No. 3. The corresponding transcripts of MP-19 were obtained from human placenta tissue and code for a protein showing considerable amino acid homology to the PP2C proteins (shown in Figure 1 deduced from SEQ ID NO. 1). The protein sequence of rabbit and human PP2C α and rat and rabbit PP2C α are described in Mann et al. (1992) Biochim. Biophys. Acta 1130, 100-104. Some typical sequence homologies, which are specific for the known PP2Cs, were also found in the MP-19 sequence. In the present invention, cloning was carried out according to the method described below. Once the DNA sequence has been cloned, the preparation of host cells
35 capable of producing the PP2C-like protein MP-19 and the production of said protein can be easily accomplished using known recombinant DNA techniques comprising constructing the expression plasmids encoding said protein and transforming a host cell with said expression plasmid, cultivating the transformant in a suitable culture medium, and recovering the product having PP2C-like activity.

Thus, the invention also relates to recombinant molecules comprising DNA sequences as described above, optionally linked to an expression control sequence. Such vectors may be useful in the production of the PP2C-like protein in stable or transiently transformed cells. Several animal, plant, fungal and bacterial systems may be employed for the transformation and subsequent cultivation process. Preferably, expression vectors which can be used in the invention contain sequences necessary for the replication in the host cell and are autonomously replicable. It is also preferable to use vectors containing selectable marker genes which can be easily selected for transformed cells. The necessary
45 operation is well-known to those skilled in the art.

It is another object of the invention to provide a host cell transformed by an expression plasmid of the invention and capable of producing a protein of the serine/threonine phosphatase family. Examples of suitable host cells include various eucaryotic and prokaryotic cells, such as *E. coli*, insect cells, plant cells, mammalian cells, and fungi such as yeast.

Another object of the present invention is to provide a PP2C-like protein or a biologically active fragment thereof encoded by the sequences described above and displaying biological features such as dependency of Mg^{2+} (or Mn^{2+}) for activity. Furthermore, the phosphatase catalyzes dephosphorylation of phosphoserine/threonine residues of proteins and peptides phosphorylated by cAMP-dependent protein kinases and protein kinase C. It is insensitive to inhibitors like okadaic acid and calyculin A, heparin and PP1 inhibitors 1 and 2. It does not attack phosphorylase α . It is inhibited by polycations and F^- ions. A preferred substrate for the PP2C-like protein is the SET protein, suggesting capacities possible relevant to therapeutically treatment of leukemia. Furthermore, the PP2C-like protein prefers basic substrates such as histones, and MBP phosphorylated by cAMP-dependent protein kinase, suggesting a special function for this phosphatase in the brain. The amino acid sequences of especially preferred PP2C-like proteins (MP-19) are shown in
55 SEQ ID NO. 2 and SEQ ID NO. 4.

It is a further aspect of the invention to provide a process for the production of PP2C-like proteins. Such a process comprises cultivating a host cell being transformed with a nucleic acid sequence of the present invention in a suitable culture medium and purifying the PP2C-like protein produced. Thus, this process allows the production of the sufficient amount of the desired protein for use in medical treatments. The host cell is obtainable from bacteria such as *Bacillus spec.* or *Escherichia coli*, from fungi such as yeast, from plants such as tobacco, potato, or *Arabidopsis*, and from animals, in particular vertebrate cell lines such as the Mo-, COS- or CHO cell line.

It is another object of the present invention to provide pharmaceutical compositions containing a therapeutically-effective amount of a PP2C-like protein of the present invention and, optionally, a pharmaceutically acceptable carrier and/or diluent, and/or agonists and/or antagonists thereof. Such a therapeutic composition can be used for the treatment of cancer such as leukemia, brain cancer, breast cancer and prostate cancer. The pharmaceutical composition according to the present invention can also be therapeutically applied for degenerative disorders of the CNS, e.g. Alzheimer's disease, Huntington's disease, Parkinson's disease, and epilepsy, and disorders of the reproductive system e.g. fertility disorders or testicular cancer. Another possible clinical application of a PP2C-like protein is the use for treatment of liver diseases, diabetes, and cystic fibrosis. The pharmaceutical composition comprising the protein of the present invention can also be used for treatment of microbial or viral infections. Another application of the pharmaceutical composition of the present invention is the usage of the protein in the regulation of spermatogenesis or the maturation of mammalian germ cells, e.g. for contraception.

Furthermore, the application of the composition is not limited to humans but can include animals, in particular domestic animals, as well.

Finally, another object of the present invention is an antibody or antibody fragment, which is capable of specifically binding to the proteins of the present invention. Methods to raise such specific antibody are known in the art. Such an antibody is preferably a monoclonal antibody. Such antibodies or antibody fragments might be useful for diagnostic methods.

The following examples illustrate in more detail the present invention, but should not be construed as limiting the invention.

Example 1

Isolation of MP-19

For the reverse transcription reaction, 5 µg total RNA (0.5 µg/µl) derived from human placenta tissue was heated for 5 minutes and cooled rapidly on ice for 5 minutes. The reverse transcription reagent mixture containing 5 µg total RNA, 38 u of RNA-guard (Pharmacia), 2.5 µg oligomer d(T)12-18 (Boehringer Mannheim), 5x reaction buffer (250 mM Tris/HCl pH 8.5, 50 mM MgCl₂, 50 mM DTT, 600 mM KCl), 10 mM of each dNTP (Pharmacia), 37.5 u of avian myeloblastosis virus reverse transcriptase (AMV, Boehringer Mannheim). The reaction mixture (20 µl) was incubated for 90 minutes at 42°C. The resulting placenta cDNA pool was stored at -20°C.

For the primary polymerase chain reaction (PCR), a placenta-derived cDNA pool was used as template in a 50 µl reaction mixture. The PCR reaction was carried out in a RoboCycler Gradient 96 (Stratagene). The amplification was performed in 1x PCR-buffer (10 mM Tris/HCl pH 8.3; 50 mM KCl; 0.001 % gelatine), 1 mM of each dNTP (Pharmacia), 100 pmol of each oligonucleotide (ALK6-N2, 5'-TT(CT)(AG)C(AGCT)AT(AGCT)ATAGAAGAAGATGA-3' and ALK6-R2, 5'-CC(AGCT)CGCCA(CT)TT(AGCT)CCCATCCA-3') and 1.5 u Taq polymerase (Perkin Elmer). The PCR reaction contained cDNA corresponding to 30 ng of total RNA as starting material. The reaction mixture was overlaid by 40 µl paraffin incubated for 180s/94°C and subjected to 30 cycles (50s/94°C, 90s/48°C, 60s/72°C) with an additional extension for 480s/72°C in the Thermocycler.

A second round of amplification was performed as described above with exception that 5 µl from the first PCR reaction was used as template DNA for the PCR. A 10 µl sample from the second PCR amplification was fractionated by electrophoresis using a 2 % agarose gel in TBE buffer. After electrophoresis amplified DNA corresponding to a molecular weight of about 600-800 bp was excised from the gel and isolated by 3x freeze/thaw cycles (-20 °C / + 37 °C) and using the DNA Purification Kit "Easy Pure" (Biozyme, Cat. no. 39001) following the instructions of the manufacturer.

The eluted DNA was amplified a third time as described in the primary PCR with exception that 3 µl of the eluted DNA, resulted from the second round of amplification, was used as template for PCR and the annealing temperature was 56 °C instead of 48 °C. After electrophoresis using a 2% agarose gel in TBE buffer, a distinct DNA band that corresponds to a molecular weight of about 700 bp was eluted with the extraction method described before. After than an additionally purification using the QIAquick 8 PCR Purification Kit (Qiagen, Cat. no. 28144) following the instructions of the manufacturer, was carried out.

Cloning of the purified DNA was established using the Original TA Cloning Kit (Invitrogen, Cat. no. K2000-40). Plasmid DNA from positive clones was isolated with the QIAwell 8 Plus Plasmid Kit (Qiagen, Cat. no. 16142) and sequenced with an automatic DNA sequencer (ALFexpress, Pharmacia). The resulting DNA sequence was analyzed by a homol-

ogy search with the blast program.

Example 2

Isolation of MP-19 full-length cDNA

Isolation of a full-length cDNA clone of MP-19 was performed with a commercial available Human Placenta cDNA Library (Stratagene, Cat. no. 937225). For screening, a labeled PCR probe was generated from MP-19 cDNA (SEQ ID NO. 1). The amplification was performed in 1x PCR-buffer (Qiagen, Germany), 1 mM of dATP, 1 mM of dCTP, 1 mM of dGTP, 0.6 mM of dTTP (Pharmacia, Germany), 0.4 mM of Digoxigenin-11-dUTP (Boehringer, Mannheim), 100 pmol of each oligonucleotide PL19-N1 (5'-GGGCAGAACTGTGCACAGGG-3') and PL19-R1 (5'-CATC GGTGACCTTGCCACC-3') and 1 u Taq DNA-polymerase (Qiagen). The PCR mix was overlaid by 40 µl paraffin oil and incubated for 180s/94 °C and subjected to 30 cycles (60s/94 °C, 60s/58 °C, 60s/72 °C) with an additional extension 180s/72 °C.

Prehybridization of plaque lift filters from cDNA library was done at 58 °C for 4 h in 0.25 M Na₂HPO₄, 7 % SDS, 1 mM EDTA, pH 7.2. Hybridization was carried out with 50 ng labeled MP19 PCR probe for 15 h under standard conditions as prehybridization was done. Filter washed 3 times (5 min, 10 min and 15 min) with 30 mM Na₂HPO₄, 0.1 % SDS at 60 °C. Detection of signals were performed with DIG Luminescent detection kit from Boehringer, Mannheim (Cat. no. 1363514). Proceed from a positive signal, clone 39-1 was isolated and sequenced. The resulting cDNA sequence from clone 39-1 conform to Bp. 387-1641 in SEQ ID NO. 3. To generate the 5' cDNA end of MP-19 full-length cDNA, 1 µg of a Lambda-DNA preparation from Human Placenta cDNA Library was subjected to PCR amplification was performed in 1x PCR-buffer (Qiagen), 1 mM of each dNTP (Pharmacia), 100 pmol of each oligonucleotide MP19-E5 (5'-GGATCCATGGGTGCCTACCTCTCCAGCCC-3') which was derived from an EST sequence (accession no. AA115688) and MP19-3 (5'-GCCTGTGTAGGCTTGGCTGTGGGGCC-3') and 1 u Taq DNA-polymerase (Qiagen). The reaction was overlaid by 40 µl paraffin oil and incubated for 180s/94 °C and subjected to 30 cycles (60 °C, 60s/74 °C, 60s/72 °C) with an additional extension for 300s/72 °C. The resulting PCR fragment was subcloned into vector pCR 2.1 (Clontech, Germany). Corresponding DNA was digested with restriction endonucleases Bam HI and Stu I. After that MP-19 Bam HI/Stu I fragment was inserted into clone 39-1. The resulted clone was named 28-9 and DNA-sequence present the full-length cDNA-sequence of MP-19 as shown in SEQ ID NO. 3. The corresponding amino acid sequence of MP-19 is shown in SEQ ID NO. 4.

Gene expression of MP-19 in human tissues

Relative expression of the MP-19 gene was determined by Northern blot analyses. A commercial available Human RNA Master Blot (Clontech, Germany, Cat. no. 7770-1, Lot no. 7090716) was hybridized with the digoxigenin labeled MP-19 PCR probe as described in: Isolation of MP-19 full-length cDNA. 50 different human tissue samples were investigated for MP-19 gene expression. Additionally 8 different negative controls from *E. coli*, yeast and human genomic DNA were applied.

Main expression of MP-19 was detected in human testis which is shown in figure 2. Lower expression of MP-19 was detected in human pituitary gland, thymus, small intestine and fetal liver. Basal expression of MP-19 was found in other human RNA samples. No hybridization signals were detected in negative controls.

Expression of MP-19 cDNA in *E. coli*

The cDNA of clone 28-9 was subcloned into the expression plasmid pQE-16 (Qiagen, Germany). This cloning strategy constituted an additional tag of 6 histidine residues at the C-terminus of MP-19. pQE-16 was digested with Bam HI and Bgl II. The 5' part of MP-19 was excised from clone 28-9 with Bam HI and Sac I. To constitute a compatible 3' end of MP-19 for cloning into plasmid pQE-16, a PCR was performed with primer MP19N-Sac I (5'-ACAGCAGAGC CAGCCAGAG-3') and MP19R-Bgl II (5'-AGATCTGTCTCGCTTGGCTTCTTCT-3') and template DNA of clone 28-9. The 9.5' and 3' end of MP-19 DNA was ligated into pQE-16 to establish MP-19 with His-tag, which was expressed in *E. coli* strain M15 (Qiagen). For recombinant expression of MP-19, cells were grown in a 5 l fermenter (Bio Console ADI 10 Applikon, Netherlands) at 37 °C in LB-Medium until an OD₆₀₀ of 2.5 was reached. After induction with 1 mM β-D-oxalactopyranoside, cells were grown for additional 4 h until OD₆₀₀ of 9.7 was reached. Cells were harvested by centrifugation at 10,000 x g for 30 min, washed once in 500 ml 1 x PBS buffer (30 min at 10,000 x g) and were frozen in liquid nitrogen at -80 °C. For preparation of MP-19 protein, 10 g cells were lysed in 100 ml lysis buffer (50 mM NaH₂PO₄, 8.0 mM NaCl, 10 mM imidazole, 100 mg lysozyme (Serva, Germany) and 50 u Benzonase (Merck, Germany)) followed by sonication 3 times with an ultrasonic processor (UP-200S, Dr. Hielscher GmbH, Germany) for 3 min at 10 Wsec⁻¹ in an ice/water bath. Cell debris was removed by centrifugation for 30 min at 4 °C and 25,000 x g.

Purification of recombinant MP-19

Recombinant expressed MP-19 was purified using Immobilized Metal Ion Affinity Chromatography (IMAC) and Reverse Phase Liquid Chromatography (RPLC). The chromatographic purification was realized using the ÄKTA-Explorer system (Pharmacia Biotech, Germany). For IMAC, a 1 ml Hi-Trap chelating column (Pharmacia Biotech) is used. Hi-Trap column was activated with 5 column volumes (cv) of 100 mM NiSO_4 , afterwards the column washed with 5 cv water to remove unbound Ni^{2+} . Column equilibration was performed with 5 cv of lysis buffer (50 mM NaH_2PO_4 , 300 mM NaCl, 10 mM Imidazole, pH 8.0). Cell lysat results from 1 g *E. coli* cells was loaded onto column. Afterwards the column washed with lysis buffer to remove unbound protein. Protein were eluted using the following gradient program. Step 1: 20 mM imidazole to 300 mM imidazole within 20 minutes, step 2: 300 mM imidazol to 500 mM imidazol within 10 minutes using buffer A (50 mM Tris, 300 mM NaCl, 20 mM Imidazole, pH 6.0) and buffer B (50 mM Tris, 300 mM NaCl, 500 mM Imidazole, pH 6.0). Flow rate of chromatography were 1 ml/min, detection were performed at 280 nm. Fractions were analyzed by immunological detection, shown in Figure 3. For further purification, positive fractions containing MP-19 were pooled and loaded onto a Resource RPC (3ml) column (Pharmacia Biotech). Column was equilibrated with buffer A (0.1 % trifluor acetic acid) and protein eluted with a linear gradient of buffer B (0.1 % TFA-90 % acetonitrile). Flow rate of chromatography were 3 ml/min, detection were performed at 215 nm.

Immunological Detection of MP-19

Immunological detection of recombinant MP-19 was performed by western blotting using a commercial available monoclonal mouse antibody against histidine-tag (Dianova, Germany, Cat. no. dia900) in combination with Western Light chemoluminescent detection system using the goat anti-mouse-AP antibody (Tropix, U.S.A.).

Activity assay for recombinant MP-19

A Serine/Threonine Phosphatase Assay System (Promega, Germany, Cat. no. V2460) was used to determine enzymatic activity of recombinant MP-19. This assay use a chemically synthesized phosphopeptide RRA(pT)VA which is a functional substrate for MP-19 phosphatase. The amount of free phosphate which is generated by MP-19 enzymatic reaction was measured by the absorbency of a molybdate:malachite green:phosphate complex (Ekman P. and Jager O. (1993). Anal. Biochem. 214, 138-141, Deana A. D. et al. (1990), Biochimica et Biophysica Acta 1051, 199-202). Assays were performed as described by the manufacturer in PPase-2C buffer (50 mM imidazole, pH 7.2, 0.2 mM EGTA, 5 mM MgCl_2 , 0.02 % β -mercaptoethanol, 0.1 mg/ml BSA). To determine background of this assay, clone pQE-16-dhfr (Clagen, Germany) was used, which is identical to pQE-MP-19 with exception that vector pQE-16 inserted a mouse dhfr gene instead of MP-19 phosphatase gene.

Results of activity assay of MP-19 shown in table 1. MP-19 has a significant activity in a MgCl_2 containing buffer, but no activity in a CaCl_2 containing buffer, which shows the requirement to Mg^{2+} . Inhibitors like okadaic acid (10 μM) shows no significant reduction of MP-19 activity. Control expression of the mouse dhfr gene shows no activity in the Serine/Threonine Phosphatase Assay System.

	A	B	C	D (Average A-C)
1	0.000	0.006	-0.006	0.000
2	0.024	0.029	0.023	0.025
3	0.304	0.291	0.298	0.298
4	0.612	0.594	0.597	0.601
5	1.080	1.140	1.137	1.119
6	0.021	0.006	0.018	0.015
7	0.025	0.043	0.018	0.029
8	0.038	0.020	0.018	0.025
9	0.030	0.012	0.013	0.018
10	0.151	0.108	0.174	0.144
11	0.146	0.147	0.139	0.144

Table 1: Activity test of MP-19

- A1-D1: Phosphate standard 0 pmol
 A2-D2: Phosphate standard 100 pmol
 A3-D3: Phosphate standard 500 pmol
 A4-D4: Phosphate standard 1000 pmol
 A5-D5: Phosphate standard 2000 pmol
 A6-D6: mouse dhfr gene with substrate (negative control)
 A7-D7: mouse dhfr gene without substrate (negative control)
 A8-D8: MP-19 with modified PPTase-2C buffer (5mM MgCl₂ is replaced by 5 mM CaCl₂) and substrate
 A9-D9: MP-19 with PPTase-2C buffer without substrate
 A10-D10: MP-19 with PPTase-2C buffer and substrate
 A11-D11: MP-19 with PPTase-2C buffer, substrate and 10 μ M okadaic acid

Annex to the description

Sequence listing

5	SEQ ID NO. 1	
10	TACGGGCGAG ACTGTCACAA GGGCCCTCCC CACAGCAAAT CTGGAGGTGG	50
	GACAGGCGAG GAACCAGGGT CCCAGGGCCT CAATGGGGAG GCAGGACCTG	100
	AGGACTCAAC TAGGGAAACT CCTTCACAAG AAAATGGCCC CACAGCCAG	150
	GCCTACACAG GCTTTTCCTC CAACTCGGAA CGTGGGACTG AAGCAGGCCA	200
15	AGTTGGTGAG CCTGGCATTG CCACTGGTGA GGCTGGGCCT TCCTGCTCTT	250
	CAGCCTCTGA CAAGCTGGCT CGAGTTGCTA AGTCCAAGTT CTTTGAGGAC	300
	AGTGAGGATG AGTCAGATGA GCGGAGGAA GAAGAGGAAG ACAGTGAGGA	350
	ATGCAGCGAG GAAGAGGATG GCTACAGCAG TGAGGAGGCA GAGAATGAGG	400
20	AAGATGAGGA TGACACCGAG GAGGCTGAAG AGGACGATGA AGAAGAAGAA	450
	GAAGAGATGA TGGTGCCAGG GATGGAAGGC AAAGAGGAGC CTGGCTCTGA	500
	CAGTGGTACA ACAGCGGTGG TGGCCCTGAT ACGAGGGAAG CAGTTGATTG	550
	TAGCCAACGC AGGAGACTCT CGCTGTGTGG TATCTGAGGC TGGCAAAGCT	600
	TTAGACATGT CCTATGATCA CAAACACAGG GATGAAGTAG AACTAGCACC	650
25	CATCAAGAAT GCTGGTGGCA AGGTACCC	678
30	SEQ ID NO. 2	
	YQQNCHKGPP HSKSGGGTGE EPGSQQLNGE AGPEDSTRET PSQENGPTAK	50
	AYTGFSSNSE RGTEAGQVGE PGIPTGEAGP SCSSASDKLP RVAKSKFFED	100
35	SEDESDEAEE EEEDSEECSE EEDGYSSEA ENEDEDDTE EAEEDEEEE	150
	EEHVPFGMEG KEEPGSDSGT TAVVALIRGK QLIVANAGDS RCVVSEAGKA	200
	LDMSYDHKPE DEVELARIKN AGGKVT	226
40		
45		
50		
55		

SEQ ID NO. 3

ATGGGTGCTAGCTCTCTCCAGCCCAACACGGTGAAGTGCCTCCGGGACCGGGGTGGGGCCCC
 GCGCCTGCGGCTGCGCTACGGCTTCTCCGCCATGCAAGGCTGGCGGCTTCCATGGAGGATG
 CTCACAACTGTATTCTCTGAGCTGGACAGTGAAGACGCCATGTTTCTGTCTACGATGGACAT
 GGAGGGGGGAAGTGTGCTTGTACTGTGCCAAATATCTTCTGATCATCAAGATCAGAA
 GGCCTACAAGGAAGGCAAGCTACAGAAGGCTTTAGAAGATGCCTTCTTGGCTATTGACGCCA
 AATTGACCACTGAAGAGTCAATTAAGAGCTGGCACAGATTGCAGGGGACCCACTGAGGAT
 GAAGATGAAAAAGAAAAAGTAGCTGATGAAGATGATGTGGACAATGAGGAGGCTGCATGCT
 GCATGAAGAGGCTACCATGACTATTSAAGAGCTGCTGACACGCTACGGGCGAGAATGTCCACA
 AGGGCCCTCCCCACAGCAATCTGGAGGTGGGACAGGGGAGGAACCGGGTCCCCAGGGCCTC
 AATGGGGAGGCGAGGACCTGAGGACTCAACTAGGGAACCTCCTTACAGAAAAATGGCCCCAC
 AGCCAAGGCCCTACACAGGCTTTTCTCCAACTCGGAACCTGGGACTGAGGCGAGGCAAGTTG
 GTGAGCCTGGCATTCCCACTGGTGAGGCTGGGCCCTTCTGCTCTCAGCCTCTGACAAGCTG
 CCTCGAGTTGCTAAGTCCAAAGTTCTTTGAGGACAGTGAGGATGAGTCAGATGAGGCGGAGGA
 AGAAGAGGAAGACAGTGAAGAAATGCAGCGAGGAAGAGGATGGCTACAGCAGTGAGGAGGCGAG
 AGAATGAGGAAGATGAGGATGACACCGAGGAGGCTGAAGAGGACGATGAAGAGGAAGAGAA
 GAGATGATGGTGCCAGGGATGGAAGGCCAAGAGGAGGCTGGCTGACAGTGGTACAACAGC
 GGTGGTGGCCCTGATACGAGGGAAGCAGTTGATTGTAGCCAACCGCAGGAGACTCTCGCTGTG
 TGGTATCTGAGGCTGGCAAGCTTTAGACATGCTCTATGATCACAACCCAGAGGATGAAGTA
 GAACTAGCACGCATCAAGAATGCTGGTGGCAAGCTCACCATGGATGGGCGAGTCAACGGGGG
 CCTCAACCTCTCCAGAGCCATTGGGAGCCACTTCTATAAGAGAAACAAGAACTGCCACCTG
 AGSAACAGATGATTTCAGCCCTTCTGACATCAAGGTGCTGACTCTGACAGCAGCATGAA
 TTCATGGTTCATTGCTGTGATGGCATCTGGAATGTGATGAGCAGCGAGGAAGTTGTAGATT
 CATTCAATCAAGATCAGCCAGCGTGATGAAAATGGGGAGCTTCGGTTATTGTATCATCATT
 TGGAAGAGCTGCTGGATCAGTGCCTGGCACCAGACACTTCTGGGATGTTACAGGCTGTGAC
 AACATGACCTGCATCATCTATTGCTTCAAGCCCCGAAACACAGCAGAGCTCCAGCAGAGAG
 TGGAAGCGCAAACTAGAGGAGGTGCTCTACTGAGGGGGCTGAAGAAAAATGCCAACAGCG
 ACAAGAAGAAGAAGGCCAAGCGGAGACTAG

SEQ ID NO. 4

MGAYLSQPNP VKCSGDEVGA PRLPLPYGFS AMGWVRVSMH DARNICPELD SETAMFSVYO
 GHGGEVVALY CAXVLPDICH DQKXVXGKHL QKALEDAFLA IDAKLTTEEV IKELACIAGR
 PTEDEDEKEK VAEEDDVONE EAALEHEAT WIEELLTRY QNCHXGPPH SKSGGCTGEE
 PGSOGLNGEA GPEDSTRETP SQENGPTAKA VTGFSNSER GTEAGQVGEPT GIPTGAGPS
 CSSASOKLPR VAKSKFFEDS EDESDEAEZE EDSSECCSE EDGYSSEAE NEDEDDTTE
 AEEDDEEEFE EMMVPGMEGK EEPGSDSGTT AVVALYRQKQ LVANAGDSR CVVSEAGKAL
 DMSYONKPED EVELARIKNA GGVKTMDOGV NGGLNLRSRI GDHYKRNKN LPPEQMISA
 LPDIFVLTLT DDHCFMVIAC DGIWNVMSQ EVDFIOSKI SQRENGELA LSSIVEELL
 DQCLAPDTSG DDTGCDNMTG IICFKPNRT AEVQPESGKR KLEZVLSTEG AEENGNSDKK
 KRAKRD

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Biopharm GmbH
(B) STREET: Czernyring 22
(C) CITY: Heidelberg
(D) COUNTRY: Germany
(F) POSTAL CODE (ZIP): 69115

(ii) TITLE OF INVENTION: Nucleic acid encoding a novel human protein phosphatase

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: EP 98107346.3

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 678 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TACGGGCAGA	ACTGTCAACA	GGGCCCTCCC	CACAGCAAA	CTGGAGGTGG	GACAGGCGAG	60
GAACCAAGGT	CCACAGGGCT	CAATGGGGAG	GCAGGACCTG	AGGACTCAAC	TAGGGAAACT	120

CTTTCACAAG AAAATGGCCC CACAGCCAAG GCCTACACAG GCTTTTCCTC CAACTGGGAA 180
 CGTGGGACTG AAGCAGGCCA AGTTGGTGAG CCTGGCAITC CCACTGGTGA GGCTGGGCTT 240
 TCCTGCTCTT CAGCCTCTGA CAAGCTGCCT CGAGTTGCTA AGTCCAAATT CTTTGAGGAC 300
 AGTGAGGATG AGTCAGATGA GCGGAGGAA GAAGAGGAAG ACAGTGAGGA ATGCAGCGAG 360
 GAAGAGGATG GCTACAGCAG TGAGGAGGCA GAGAATGAGG AAGATGAGGA TGACACCGAG 420
 GAGGCTGAAG AGGACGATGA AGAAGAAGAA GAAGAGATGA TGSTGCCAGG GATGGAAGGC 480
 AAAGAGGAGC CTGGCTCTGA CAGTGGTACA ACAGCGGTGG TGGCCCTGAT ACGAGGGAAG 540
 CAGTTGATIG TAGCCCAACG AGGAGACTCT CGCTGTGTGG TATCTGAGGC TGGCAAAAGCT 600
 TTAGACATGT CCTATGATCA CAAACCAGAG GATGAAGTAG AACTAGCACG CATCAAGAAT 660
 GCTGTTGGCA AGGTCACC 678

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 226 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Tyr Gly Gln Asn Cys His Lys Gly Pro Pro His Ser Lys Ser Gly Gly
 1 5 10 15
 Gly Thr Gly Glu Glu Pro Gly Ser Gln Gly Leu Asn Gly Glu Ala Gly
 20 25 30
 Pro Glu Asp Ser Thr Arg Glu Thr Pro Ser Gln Glu Asn Gly Pro Thr
 35 40 45
 Ala Lys Ala Tyr Thr Gly Phe Ser Ser Asn Ser Glu Arg Gly Thr Glu

	50	55	60
5	Ala Gly Gln Val	Gly Glu Pro Gly Ile	Pro Thr Gly Glu Ala Gly Pro
	65	70	75 80
	Ser Cys Ser Ser	Ala Ser Asp Lys Leu	Pro Arg Val Ala Lys Ser Lys
		85	90 95
10	Phe Phe Glu Asp	Ser Glu Asp Glu Ser	Asp Glu Ala Glu Glu Glu
		100	105 110
	Glu Asp Ser Glu	Glu Cys Ser Glu Glu	Asp Gly Tyr Ser Ser Glu
		115	120 125
15	Glu Ala Glu Asn	Glu Glu Asp Glu Asp	Thr Glu Glu Ala Glu Glu
		130	135 140
	Asp Asp Glu Glu	Glu Glu Glu Met Met	Val Pro Gly Met Glu Gly
		145	150 155 160
20	Lys Glu Glu Pro	Gly Ser Asp Ser Gly Thr	Ala Val Val Ala Leu
		165	170 175
	Ile Arg Gly Lys	Gln Leu Ile Val Ala	Asn Ala Gly Asp Ser Arg Cys
		180	185 190
25	Val Val Ser Glu	Ala Gly Lys Ala Leu	Asp Met Ser Tyr Asp His Lys
		195	200 205
	Pro Glu Asp Glu	Val Glu Leu Ala Arg	Ile Lys Asn Ala Gly Gly Lys
		210	215 220
30	Val Thr		
	225		

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1641 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: human placenta

EP 0 874 052 A2

(X1) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

	ATGGGTGCTT ACCTCTCCCA GCCCAACACG GTGAAGTGCT CCGGGGACGG GGTGGGCGCC	60
5	CCGCGCCTGC CGCTGCCCTA CGGCTTCTCC GCCATGCAAG GCTGGCGCGT CTCATGGAG	120
	GATGCTCACA ACTGTATTCC TGAGCTGGAC AGTGAGACAG CCAATGTTTC TGCTACGAT	180
	GGACATGGAG GGGAGGAAGT TGCCTTGTAC TGTGCCAAAT ATCTTCCTGA TATCATCAAA	240
10	GATCAGAAGG CCTACAAGGA AGGCAAGCTA CAGAAGGCTT TAGAAGATGC CTCTTGGCT	300
	ATTGACGCCA AATTGACCAC TGAAGAAGTC ATTAAGAGAG TGGCACAGAT TGCAGGGCGA	360
	CCCACTGAGG ATGAAGATGA AAAAGAAAAA GTAGCTGATG AAGATGATGT GGACAATGAG	420
15	GAGGCTGCAC TGCTGCATGA AGAGGCTACC ATGACTATTG AAGAGCTGCT GACACGCTAC	480
	GGGCAGAACT GTCACAAGGG CCTCCCCAC AGCAAACTCTG GAGGTGGGAC AGGCGAGGAA	540
	CCAGGGTCCC AGGGCCTCAA TGGGGAGGCA GGACCTGAGG ACTCAACTAG GGAAACTCCT	600
20	TCACAAGAAA ATGGCCCCAC AGCCAAGGCC TACACAGGCT TTCTCTCCAA CTCGGAACGT	660
	GGGACTGAGG CAGGCCAAGT TGGTGAGCCT GGATTCCTCA CTGGTGAGGC TGGGCTTCC	720
	TGCTCTTCAG CCTCTGACAA GCTGCTCGA GTTGCTAAGT CCAAGTTCTT TGAGGACAGT	780
25	GAGGATGATG CAGATGAGGC GGAGGAAGAA GAGGAAGACA GTGAGGAATG CAGCGAGGAA	840
	GAGGATGGCT ACAGCAGTGA GGAGGCAGAG AATGAGGAAG ATGAGGATGA CACCGAGGAG	900
	GCTCAAGAGG ACGATGAAGA AGAAGAAGAA GAGATGATGG TGCCAGGGAT GGAAGGCAAA	960
30	GAGGAGCCTG GCTCTGACAG TGGTACAACA GCGGTGGTGG CCTGTATACG AGGGAAGCAG	1020
	TTGATTGTAG CCAACGCAGG AGACTCTCGC TGTGTGTAT CTGAGGCTGG CAAAGCTTTA	1080
	GACATGTCTT ATGATCACAA ACCAGAGGAT GAAGTAGAAC TAGCACGCAT CAAGAATGCT	1140
35	GGTGGCAAGG TCACCATGGA TGGGCGAGTC AACGGGGGCG TCAACCTCTC CAGAGCCATT	1200
	GGGGACCACT TCTATAAGAG AAACAAGAAC CTGCCACCTG AGGAACAGAT GATTTCAGCC	1260
40	CTTCTGACA TCAAGGTGCT GACTCTCACT GACGACCATG AATTCTGCTT CATTGCCTGT	1320
	GATGGCATCT GGAATGTGAT GAGCAGCCAG GAAGTTGTAG ATTTCATTCA ATCAAGATC	1380

AGCCAGCGTG ATGAAAATGG GGAGCTTCGG TTATTGTGTCAT CCATTGTGGA AGAGCTGCTG 1440
 GATCAGTGCC TGGCACCAGA CACTTCTGGG GATGGTACAG GGTGTGACAA CATGACCTGC 1500
 ATCATCATTT GTTCAAGCC CGGAAACACA GCAGAGCTCC AGCCAGAGAG TGGCAAGCGA 1560
 AAAGTAGAGG AGGTGCTCTC TACTGAGGGG GCTGAAGAAA ATGGCAACAG CGACAAGAAG 1620
 AAGAAGGCCA AGCGAGACTA G 1641

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 546 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: human placenta

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Gly Ala Tyr Leu Ser Gln Pro Asn Thr Val Lys Cys Ser Gly Asp
 1 5 10 15
 Gly Val Gly Ala Pro Arg Leu Pro Leu Pro Tyr Gly Phe Ser Ala Met
 20 25 30
 Gln Gly Trp Arg Val Ser Met Glu Asp Ala His Asn Cys Ile Pro Glu
 35 40 45
 Leu Asp Ser Glu Thr Ala Met Phe Ser Val Tyr Asp Gly His Gly Gly
 50 55 60
 Glu Glu Val Ala Leu Tyr Cys Ala Lys Tyr Leu Pro Asp Ile Ile Lys
 65 70 75 80
 Asp Gln Lys Ala Tyr Lys Glu Gly Lys Leu Gln Lys Ala Leu Glu Asp
 85 90 95
 Ala Phe Leu Ala Ile Asp Ala Lys Leu Thr Thr Glu Glu Val Ile Lys
 100 105 110

Glu Leu Ala Gln Ile Ala Gly Arg Pro Thr Glu Asp Glu Asp Glu Lys
 115 120 125
 5 Glu Lys Val Ala Asp Glu Asp Val Asp Asn Glu Glu Ala Ala Leu
 130 135 140
 Leu His Glu Glu Ala Thr Met Thr Ile Glu Glu Leu Leu Thr Arg Tyr
 145 150 155 160
 10 Gly Gln Asn Cys His Lys Gly Pro Pro His Ser Lys Ser Gly Gly Gly
 165 170 175
 Thr Gly Glu Glu Pro Gly Ser Gln Gly Leu Asn Gly Glu Ala Gly Pro
 180 185 190
 15 Glu Asp Ser Thr Arg Glu Thr Pro Ser Gln Glu Asn Gly Pro Thr Ala
 195 200 205
 20 Lys Ala Tyr Thr Gly Phe Ser Ser Asn Ser Glu Arg Gly Thr Glu Ala
 210 215 220
 Gly Gln Val Gly Glu Pro Gly Ile Pro Thr Gly Glu Ala Gly Pro Ser
 225 230 235 240
 25 Cys Ser Ser Ala Ser Asp Lys Leu Pro Arg Val Ala Lys Ser Lys Phe
 245 250 255
 Phe Glu Asp Ser Glu Asp Glu Ser Asp Glu Ala Glu Glu Glu Glu Glu
 260 265 270
 30 Asp Ser Glu Glu Cys Ser Glu Glu Glu Asp Gly Tyr Ser Ser Glu Glu
 275 280 285
 35 Ala Glu Asn Glu Glu Asp Glu Asp Asp Thr Glu Glu Ala Glu Glu Asp
 290 295 300
 Asp Glu Glu Glu Glu Glu Glu Met Met Val Pro Gly Met Glu Gly Lys
 305 310 315 320
 40 Glu Glu Pro Gly Ser Asp Ser Gly Thr Thr Ala Val Val Ala Leu Ile
 325 330 335
 Arg Gly Lys Gln Leu Ile Val Ala Asn Ala Gly Asp Ser Arg Cys Val
 340 345 350
 45 Val Ser Glu Ala Gly Lys Ala Leu Asp Met Ser Tyr Asp His Lys Pro
 355 360 365
 Glu Asp Glu Val Glu Leu Ala Arg Ile Lys Asn Ala Gly Gly Lys Val
 370 375 380
 50
 55

Thr Met Asp Gly Arg Val Asn Gly Gly Leu Asn Leu Ser Arg Ala Ile
 385 390 395 400
 5 Gly Asp His Phe Tyr Lys Arg Asn Lys Asn Leu Pro Pro Glu Glu Gln
 405 410 415
 Met Ile Ser Ala Leu Pro Asp Ile Lys Val Leu Thr Leu Thr Asp Asp
 420 425 430
 10 His Glu Phe Met Val Ile Ala Cys Asp Gly Ile Trp Asn Val Met Ser
 435 440 445
 Ser Gln Glu Val Val Asp Phe Ile Gln Ser Lys Ile Ser Gln Arg Asp
 450 455 460
 15 Glu Asn Gly Glu Leu Arg Leu Leu Ser Ser Ile Val Glu Glu Leu Leu
 465 470 475 480
 20 Asp Gln Cys Leu Ala Pro Asp Thr Ser Gly Asp Gly Thr Gly Cys Asp
 485 490 495
 Asn Met Thr Cys Ile Ile Ile Cys Phe Lys Pro Arg Asn Thr Ala Glu
 500 505 510
 25 Leu Gln Pro Glu Ser Gly Lys Arg Lys Leu Glu Glu Val Leu Ser Thr
 515 520 525
 Glu Gly Ala Glu Glu Asn Gly Asn Ser Asp Lys Lys Lys Ala Lys
 530 535 540
 30 Arg Asp
 545
 35

Claims

- 40 1. A nucleic acid comprising a nucleotide sequence encoding a human serine/threonine phosphatase or a functional
 fragment thereof that is capable of dephosphorylating serine or threonine residues, wherein the nucleotide
 sequence comprises:
 45 (a) the nucleotides as shown in SEQ ID NO. 1 or SEQ ID NO. 3; or
 (b) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequence of (a); or
 (c) an allelic derivative of the sequences of (a) or (b); or
 50 (d) a DNA sequence which is capable of hybridizing to the sequences in (a), (b) and (c), and encoding a protein
 containing the amino acid sequence as depicted in SEQ ID NO. 2 or SEQ ID NO. 4; or
 (e) a nucleotide sequence which is capable of hybridizing to the DNA sequences in (a), (b), (c) and (d), and
 encoding a protein having essentially the same biological properties as the protein defined in (d).
 55 2. The nucleic acid according to claim 1, wherein the nucleotide sequence is a vertebrate DNA sequence, a mamma-
 lian sequence, preferably a primate, human, porcine, or rodent, preferably a rat or rabbit, DNA sequence.

3. A recombinant molecule comprising a nucleic acid according to claim 1 or 2.
4. The recombinant molecule according to claim 3, wherein said nucleic acid sequence is functionally linked to an expression-control sequence.
5. A host containing the nucleic acid according to claim 1 or 2, or the recombinant molecule according to claim 3 or 4.
6. The host according to claim 5, which is a bacterium, a fungus, a plant cell, an animal or a human cell.
7. A process for the production of a PP2C-like protein comprising cultivating a host according to claim 5 or 6 and recovering said PP2C-like protein from the culture.
8. A PP2C-like protein or a biologically active fragment thereof encoded by a nucleic acid according to claim 1 or 2 or by a recombinant molecule according to claim 3 or 4.
9. The protein according to claim 8, comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4.
10. An agonist as a substitute for the protein of claim 8 or 9.
11. An antagonist directed to the protein of claim 8 or 9.
12. A pharmaceutical composition containing the protein or a biologically active fragment thereof according to claim 8 and 9 or the agonist according to claim 10 or the antagonist according to claim 11, and optionally, a pharmaceutically acceptable carrier and/or diluent.
13. The pharmaceutical composition according to claim 12 for the treatment of leukemia, brain cancer, breast cancer, prostate cancer, Alzheimer's disease, Huntington's disease, Parkinson's disease, and epilepsy, and of disorders of the reproductive system, or for the regulation of spermatogenesis or the maturation of mammalian germ cells.
14. An antibody or antibody fragment which is capable of specifically binding to the protein of claim 8 and 9 or to the agonist of claim 10 or to the antagonist of claim 11.
15. The antibody according to claim 14, which is a monoclonal antibody.
16. Use of an antibody or antibody fragment according to claim 14 or 15 for detecting the protein or a biologically active fragment thereof as defined in claim 8 or 9.
17. A diagnostic kit containing the agonist according to claim 10 or the antagonist according to claim 11 or the antibody or antibody fragment according to claim 14 or 15.

Fig. 1

MP19-PCR	YGCNCHKGPP	HSKSGGGTGE	SPGSQGLNGE	AGVEDSTRET	PSQENGPTAX	50
PP2C-Human	MGAFLDKPKM	EKHNAQQQG-	----NGLRYG	LSSHQGNRVE	MEDAHTAVIG	45
PP2C-Rabbit	MGAFLDKPKM	EKHNAQQQG-	----NGLRYG	LSSHQGNRVE	MEDAHTAVIG	45
PP2C-Rat	MGAFLDKPKM	EKHNAQQQG-	----NGLRYG	LSSHQGNRVE	MEDAHTAVIG	45
	*	*	*	*	*	
MP19-PCR	AYTGFSSMSE	RGTEAGQVGE	PGIPTCEAGP	SCSSASDKLP	RVAKSKFFED	100
PP2C-Human	LPSCLETWSF	FAVYDGHAG-	-----SQVAX	YCC--EHLDD	HITNNQDFKG	87
PP2C-Rabbit	LPSCLETWSF	FAVYDGHAG-	-----SQVAX	YCC--EHLDD	HITNNQDFKG	87
PP2C-Rat	LPSCLETWSF	FAVYDGHAG-	-----SQVAX	YCC--EHLDD	HITNNQDFKG	87
	*	*	*	*	*	
MP19-PCR	SEDESDEAE	EEDSERCSE	EEDGYSSEEA	ENEEDDEDOTE	EAEEDDEEEE	150
PP2C-Human	SAGAP-SVEN	VKNGI-----	-RTGF-----	-----LEID		109
PP2C-Rabbit	SAGAP-SVEN	VKNGI-----	-RTGF-----	-----LEID		109
PP2C-Rat	SAGAP-SVEN	VKNGI-----	-RTGF-----	-----LEID		109
	*	*	*	*	*	
MP19-PCR	EEMMVPGNEG	KEEPGSDSGT	TAVVALIRGK	QLIVANAGDS	RCVVSEAGKA	200
PP2C-Human	ENMRV--HSE	KKHGADRSQS	TAVGVLSLQ	HTYFINGGDS	ROLLCRNRKV	157
PP2C-Rabbit	ENMRV--HSE	KKHGADRSQS	TAVGVLSLQ	HTYFINGGDS	ROLLCRNRKV	157
PP2C-Rat	ENMRV--HSE	KKHGADRSQS	TAVGVLSLQ	HTYFINGGDS	ROLLCRNRKV	157
	*	*	*	*	*	
MP19-PCR	LDMSYDHKPE	DEVELARIKN	AGGKVT			226
PP2C-Human	HFFTQDHKPS	NPLEKERIQN	AGGSVM			183
PP2C-Rabbit	HFFTQDHKPS	NPLEKERIQN	AGGSVM			183
PP2C-Rat	HFFTQDHKPS	NPLEKERIQN	AGGSVM			183
	****	*	**	***	*	

Figure 2

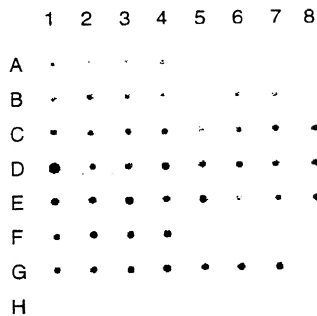
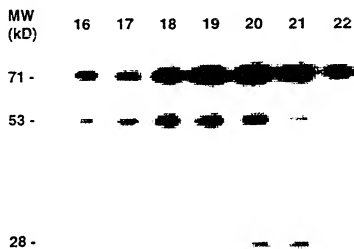


Figure 3





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(54) Nucleic acid encoding a human protein phosphatase

(57) The present invention relates to nucleic acids encoding a novel human protein phosphatase of the family of protein serine/threonine phosphatases. In particular, it relates to novel DNA sequences encoding serine/threonine protein phosphatase, to expression plasmids containing said nucleic acids, to host organisms transformed by said expression plasmids, to the production of said protein by culturing said transformant, to antibodies specifically binding to said phosphatase and to agonists and/or antagonists for said protein, and to antisense MP-19 nucleic acid. Furthermore, the invention relates to serine or threonine residues and epitopes comprising said residues dephosphorylated by said protein and pharmaceutical compositions comprising said protein or agonists or antagonists thereof for the treatment of diseases influenced by changes in phosphorylation which controls e.g. cell proliferation and/or differentiation, to diagnostic kits and to *in vitro* diagnostic methods for the detection of phosphorylation dependent diseases such as e.g. cancer.

Fig. 1

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PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention EP 98 10 7346
shall be considered, for the purposes of subsequent
proceedings, as the European search report

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (INCL. 6)
X	EMBL DATABASE ENTRY MM42383, ACCESSION NUMBER U42383, 8 June 1996, XP002085147 * abstract * - & GUTHRIE M.A. ET AL: "Induction of expression of growth related genes by FGF-4 in mouse fibroblasts" ONCOGENE, vol. 12, no. 6, 21 March 1996, pages 1267-1278, XP002035691	1-8	C12N15/55 C12N5/16 A61K38/46 C07K16/40 G01N33/577
X	"Database EMBL, Entry BTU81159, Accession number U81159 29. December. 1996" EMBL NUCLEOTIDE SEQUENCE. XP002035695 * abstract * - & Y. WANG ET AL: "A Mg ²⁺ -dependent, Ca ²⁺ -inhibitable Serine/threonine protein phosphatase from bovine brain" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 43, 27 October 1995, pages 25607-25612, XP002085148 MD US ---	1-8	TECHNICAL FIELD SEARCHED (INCL. 6) C12N
-/-			

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely:

1-9

Claims searched incompletely:

12-17

Claims not searched:

10-11

Reason for the limitation of the search:

Remark: Claims 10-11 completely and 12-17 partially are not searchable because neither an antagonist nor an agonist of the human serine/threonine phosphatase have been described.

Name of search

Date of completion of the search

Examiner

THE HAGUE

20 November 1998

LE CORNEC N.D.R.

CATEGORY OF CITED DOCUMENTS

- X: particularly relevant if taken alone
- Y: particularly relevant if combined with another document of the same category
- A: technological background
- C: non-written disclosure
- P: intermediate disclosure

- T: theory or principle underlying the invention
- E: earlier patent document, but published on, or after the filing date
- D: document cited in the application
- L: document cited for other reasons

& : member of the same patent family, corresponding document

EP 0 874 052 A3 (PCT/FR97/00001)

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PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 98 10 7346

DOCUMENTS CONSIDERED TO BE RELEVANT			Relevant to claim	CLASSIFICATION OF THE APPLICATION (INCL.9)
Category	Citation of document with indication, where appropriate, of relevant passages			
A	WO 97 10796 A (RAMOT-UNIVERSITY AUTHORITY) 27 March 1997 * claims; examples 2,3 *	---	1-9, 12-17	TECHNICAL FIELD SEARCHED (INCL.8)
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